Second Report to the Royal Society Water Research Committee. The Vitality and Virulence of Bacillus anthracis *and its Spores in Potable Waters.*

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Introduction.

In our First Report we endeavoured to give a concise account of the more important work which has been done on the bacteriology of water, and devoted special attention to those investigations which have thrown any light on the vitality of pathogenic bacteria when submerged in aqueous media of various kinds and under various circumstances. We showed what a very large amount of scientific labour has already been bestowed on this comparatively new subject; and we as far as possible sifted this accumulated material, collecting those facts which may be reasonably regarded as definitely proved, and separating them from those which are more or less uncertain, problematical, or contradictory. The results of this critical analysis we embodied in a number of conclusions, to be found on pp. 124—219 of our Report, and to which we would again refer the reader.

In this summary and conclusions we have prominently pointed out that the information which is at present most urgently required from a practical point of view is with regard to the manner in which the vitality of pathogenic bacteria is affected by the presence of non-pathogenic or saprophytic forms. Although several investigators have already approached this question, we were obliged to indicate that their results have to be received with much caution, in consequence of the great experimental difficulties which attach to this inquiry, and which we were of opinion had not in some cases been adequately taken into account to prevent misleading results being obtained.

It was owing to our consciousness of these experimental difficulties that we resolved to confine our own investigations in the first instance to the study of such a pathogenic form as would permit of these difficulties being reduced to a minimum, and to this end we naturally selected the *Bacillus anthracis*. But in so doing we also had a second object in view, for, inasmuch as the spores of this bacillus are among the hardiest forms of pathogenic organisms, their deportment under any particular conditions is of peculiar interest as exhibiting the limit of endurance which has to be taken into consideration in dealing with pathogenic bacteria. Thus, conditions which are found to be fatal to anthrax spores may in general be assumed to be *a fortiori* fatal to other pathogenic forms. Again, we have in the case of anthrax the possibility of determining in a much more decisive manner than with other forms the influence which conditions exert on the virulence of the organism.

The Vitality of Bacillus anthracis *and its Spores in Water.*

As indicated in our First Report, this question has already occupied the attention of a number of investigators. In some cases the bacilli* free from spores, in other cases sporiferous bacilli, have been employed; again, in some experiments sterile, and in others unsterilised, waters have been used, whilst the temperature at which the waters were maintained during the experiments has also been varied.

Thus, Wolffhügel and Riedel ('Arbeiten a. d. Kais. Gesundheitsamte,' vol. 1, 1886, p. 455) introduced the bacilli, which may possibly have contained spores,† as they were taken from a gelatine culture, into sterile water kept at 35° C., and obtained abundant multiplication, whilst when similar bacilli were placed in water at 7—10° C. their presence was no longer demonstrable by culture after two days, although a few bacilli (or spores) must still have been present in a virulent condition, as of four mice, each inoculated with $\frac{1}{3}$ c.c. of the water, one died of anthrax. The water employed in these experiments was primarily the polluted liquid found in the River Panke at Berlin, although the results were substantially similar when this water was diluted with ten times its volume of distilled water. These investigators made no experiments with unsterilised water.

* We adopt the following terminology throughout:—*Asporogenous* means incapable of developing spores; *sporiferous bacilli* are bacilli actually containing spores, whereas *vegetative bacilli* are bacilli free of spores, though not necessarily incapable of developing them later.

† Gelatine cultures do not develop spores very rapidly, so that it is by no means certain that spores were introduced; however, these observers added so much gelatine that we cannot attach much value to the conclusions.

We may with advantage quote their experiments in full in the following tables (pp. 167 and 168).

Their results are in nearly all important respects entirely out of harmony with those of Meade Bolton, to whose experiments we may in the next instance refer.

Meade Bolton ('Zeitsch. f. Hygiene,' vol. 1, 1886, p. 76) found that on introducing anthrax bacilli presumed to be devoid of spores into ordinary drinking water (Göttingen water supply) sterilised by steam and kept at 20° C., they were no longer demonstrable by culture after 6 days, whilst at 35° C. the same result was obtained in 55 hours. On the other hand, when he employed sporiferous anthrax bacilli, they were still demonstrable after 90 days at 20° C., both in sterile distilled and in sterile well water of bad quality, whilst at 35° C. in the same waters the organisms disappeared between the 30th and the 90th day as tested by cultivation. The waters were not tested for virulence by inoculating animals, nor does it appear whether or no in these latter experiments there was any multiplication of the anthrax bacilli, as an uncountable number were in the first instance introduced into the water. It will be most convenient to quote Meade Bolton's tabulated results *in extenso* (see p. 169).

Results in substantial agreement with those of Meade Bolton have also been recorded by Koch (Gärtner-Tiemann's book, p. 585) and Naegeli, who both state that the spores of anthrax preserve their vitality in distilled water for upwards of one year. Hochstetter ('Arbeiten a. d. Kais. Gesundheitsamte,' vol. 2, p. 1), again, found the anthrax bacilli free from spores to persist both in sterilised distilled and in sterilised drinking water for 3 days only at the outside (in some cases they actually disappeared in a quarter of an hour), whilst the sporiferous bacilli were still alive and virulent after 154 days in the same waters as well as in unsterilised seltzer water. (For detailed table of results, see pp. 170 and 171.)

Similarly again Hueppe ('Journ. f. Gasbeleucht.,' 1887, p. 129) found the anthrax bacilli no longer demonstrable on the fifth day in sterilised drinking water kept at 16° C.

Straus and Dubarry ('Arch. de Méd. Expér.,' 1889; 'Ann. de l'Inst. Pasteur,' vol. 4, pp. 109—124), on the other hand, found even the bacilli free from spores to retain their vitality in sterile drinking water at 20° C. for 28 days in one case and 65 days in another, and they proved that when such sporeless bacilli are introduced into distilled water they can form spores and persist for upwards of 131 days. The greater longevity of the anthrax bacilli in the hands of these investigators is doubtless due to their having employed a more sensitive method of cultivation than the others to whom we have referred, for, instead of simply submitting the waters to plate cultivation in the ordinary way, they first added broth to the water, so as to en-

Wolffhügel and Riedel's Experiments on the Vitality of Anthrax in Water. February, 1885.

Description.	Source of material.	Temperature in Centigrade degrees at which kept.	Volume of water used for cultivation.	Number of colonies found on gelatine plates prepared from the water infected. Directly after.	1 day.	2 days.	3 days.	4 days.	10 days.	15 days.
River Panke, unfiltered, 10 c.c.	Anthrax from gelatine culture, 1 needle-loop	35	c.c. $\frac{5}{500}$	420	8,000	34,800	..	..	..	..
,, ,,	Anthrax from gelatine culture, 1 needle-point	35	$\frac{5}{500}$	0	4	..	4,900	..	5,600	..
River Panke, unfiltered, 10 c.c. diluted with distilled water (1 : 10)	Anthrax from gelatine culture, 1 needle-loop	16	$\frac{5}{500}$	600	4,000	12,500	..	..	4,860	..
River Panke, filtered, 10 c.c.	Anthrax from gelatine culture, 1 needle-point	35	$\frac{5}{500}$	0	0	..	1,040	2060	..	7,560
River Panke, filtered, 10 c.c., diluted with distilled water (1 : 1)	Anthrax from gelatine culture, 1 needle-point	16	$\frac{5}{500}$	0	6	..	..	350	..	1,550

Wolffhügel and Riedel's Experiments on the Vitality of Anthrax in Water. February, 1885.

Description.	Source of material.	Temperature in Centigrade degrees at which kept.	Volume of water used for cultivation.	Number of micro-organisms found on gelatine plates prepared from the infected water.						
				Directly after.	1 day.	2 days.	3 days.	4 days.	10 days.	15 days.
River Panke, unfiltered, 10 c.c.	Anthrax from gelatine culture, 1 needle-point	35	c.c. $\frac{5}{500}$	42	0	12,600	..	..	..	..
			$\frac{1}{500}$	..	..	..	..	..	..	..
,, ,,	,, ,,	7—10	$\frac{5}{500}$	5	0	0	0	0	*	..
			$\frac{1}{500}$	..	..	..	..	..	..	..
River Panke, unfiltered, 10 c.c., diluted with distilled water (1 : 10)	,, ,,	35	$\frac{5}{500}$	49	9	10,000	12,500	14,600	8,750	12,000
			$\frac{1}{500}$	..	..	..	..	9,450	3,150	21,000
,, ,,	,, ,,	7—10	$\frac{5}{500}$	92	0	0	0	0	*	..
			$\frac{1}{500}$	..	..	..	..	..	..	..
River Panke, filtered, 10 c.c.	,, ,,	35	$\frac{5}{500}$	5	600	10,400	14,000	27,000	44,000	16,000
			$\frac{1}{500}$	..	..	..	..	7,000	..	4,800
,, ,,	,, ,,	7—10	$\frac{5}{500}$	4	1	3	0	0	*	..
			$\frac{1}{500}$	..	..	..	..	..	..	..
River Panke, filtered, 10 c.c., diluted with distilled water (1 : 10)	,, ,,	35	$\frac{5}{500}$	6	410	11,790	11,900	15,000	36,000	10,000
			$\frac{1}{500}$	..	..	..	..	6,300	8,000	2,800
,, ,,	,, ,,	7—10	$\frac{5}{500}$	28	7	3	0	0	†	..
			$\frac{1}{500}$	..	..	..	..	..	..	..

* On the 9th day $\frac{1}{3}$ c.c. was subcutaneously injected into a mouse. It remained alive.

† On the 9th day $\frac{1}{2}$ c.c. was subcutaneously injected into a mouse. It died after $4\frac{1}{2}$ days.

Meade Bolton's Experiments on the Vitality of Anthrax in Water, 1886.

Nature of material.	Temperature in Centigrade degrees.	Number of colonies obtained from 1 c.c. of water.		
		Directly after inoculation.	Hours. 55	Days. 6
Sporeless Anthrax.				
In ordinary tap-water....................	—	7740	..	..
,, ,, ...	+ 20° C.	..	332	0
,, ,, ...	—	..	..	..
,, ,, ...	35° C.	..	0	

Anthrax Spores. Number of Colonies obtained from 1 c.c. of Water.

Description of water.	Temperature in Centigrade degrees.	Directly after inoculation.	2—5 days.	5—10 days.	10—20 days.	20—30 days.	90 days.
Distilled water.........	+20	Innumerable	..	..	Innumerable	1680	..
,, ,, ...	,,	,,	..	..	,,	..	Innumerable
Polluted well water (filtered	,,	,,	..	..	,,	Innumerable	..
Polluted well water (unfiltered)	,,	,,	..	..	,,	,,	Innumerable

Hochstetter's Experiments on the Vitality of Anthrax in various Waters.

Source of material.	Nature of the water used.	Temperature at which the water was preserved.	Number of flasks or bottles inoculated.	Number of hours or days after which the examination was made.	Table showing the number of times the flasks or bottles were examined. I.	II.	III.	IV.	V.	VI.	VII.	VIII.	IX.	X.	XI.	XII.	Longest vital duration. Hrs.	Longest vital duration. Days.	Shortest vital duration. Hrs.	Shortest vital duration. Days.
Anthrax Bacilli. Juices from the organs of a guinea pig, dead 4 days after inoculation with anthrax.	Seltzer water	14—18° C.	3	Hours	*½	..	..	..	..	..	..	..	..	..	..	..	..	..	½	..
				Days	..	*1	*3	..	..	..	..	..	..	..	..	..				
	Distilled water	,,	2	Hours	*1	..	..	..	..	..	..	..	..	..	..	..	..	..	1	..
				Days	..	*1	..	..	..	..	..	..	..	..	..	..				
Anthrax Bacilli. Juices from the organs of a guinea pig, killed after 2 days whilst suffering from very pronounced anthrax.	Seltzer water	16—20° C.	3	Hours	*¼	..	..	..	..	..	..	..	..	..	..	..	..	..	¼	..
				Days	..	*1	*2	..	..	..	..	..	..	..	..	..				
	Berlin water supply	,,	4	Hours	½[1]	..	..	..	..	..	..	..	..	..	..	..	..	1	..	2
				Days	*16	1[2]	*2	*13	..	..	..	..	..	..	..	..				
					..	*16	..	..	..	..	..	..	..	..	..	..				
	Distilled water	,,	4	Hours	½[2]	..	..	..	..	..	..	..	..	..	..	..	½	..	..	1
				Days	..	*1	*2	*13	..	..	..	..	..	..	..	..				
Anthrax Bacilli. Juices from the organs of a guinea pig, dead 2 days after inoculation with anthrax.	Seltzer water	11—13° C.	6	Hours	1[3]	*2[4]	..	..	..	..	..	..	..	..	..	..	1	..	2	..
				Days	..	..	*1	*3[5]	*3	*7	..	..	..	..	..	..				
	Berlin water supply	,,	5	Hours	¼	2½	..	..	..	..	..	..	..	..	..	..	..	3	..	7
				Days	..	..	1	3	*7	..	..	..	..	..	..	..				
	Distilled water	,,	5	Hours	½	2½	..	..	..	..	..	..	..	..	..	..	..	3	..	7
				Days	..	..	1	3	*7	..	..	..	..	..	..	..				

Anthrax Bacilli.																				
Juices from the organs of a guinea pig, dead 2 days after inoculation with anthrax.	Seltzer water	13—16° C.	3	Hours	1[6]	..	..	..	..	..	..	..	..	..	..	..	1	..	..	1
				Days	..	*1[5]	*1	..	..	..	..	..	..	..	..	..				
	Berlin water supply	„	3	Hours	1	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..
				Days	..	1	1	..	..	..	..	..	..	..	..	..				
	Distilled water	„	2	Hours	1	..	..	..	..	..	..	..	..	..	..	..	..	1	..	..
				Days	..	1	1	..	..	..	..	..	..	..	..	..				
Anthrax Spores.																				
Potato cultivation inoculated from the juices of a guinea pig, dead 2 days after injection with anthrax. The culture was kept for 5 days in the incubator at a temperature of 30° C.	Seltzer water	13—18° C. (bottles II—VI) 18—20° C. (bottles VII, VIII)	8	Hours	$\frac{1}{4}$	..	..	..	..	..	..	..	..	..	..	..	..	154	..	..
				Days	..	1	7	14	21	28[7]	64[8]	154[9]	..	..	..	..				
	Berlin water supply		8	Hours	$\frac{1}{4}$	..	..	..	..	..	..	..	..	..	..	..	..	154	..	..
				Days	..	1	7	14	21	28	64	154[10]	..	..	..	..				
	Distilled water		8	Hours	$\frac{1}{4}$	..	..	..	..	..	..	..	..	..	..	..	..	154	..	..
				Days	..	1	7	14	21	28	64	154[10]	..	..	..	..				

Note.—Those figures preceded by an asterisk (*) indicate that at the time of investigation the organisms were dead.

1 A mouse inoculated from a colony off a gelatine plate poured from this water died of anthrax after 2 days.

2 „ „ „ „ „ „ „ „ „ 4 „

3 Guinea pigs inoculated with 4 c.c. of this infected seltzer water, subcutaneously injected, died after three days of anthrax; moreover, guinea pigs inoculated from a colony off a gelatine plate poured from this water died after two days of anthrax.

4 Bottle was kept in a room at a temperature of 18—20° C.

5 Guinea pigs inoculated with 4 c.c. of this infected seltzer water, subcutaneously injected, remained healthy.

6 „ „ „ 2 c.c. „ „ „ „ „ died after 3 days of anthrax.

7 „ „ „ 1 c.c. „ „ „ „ „ „ 2 „ „

8 „ „ „ 3 c.c. „ „ „ „ „ „ 2 „ „

9 „ „ „ 2 c.c. „ „ „ „ „ „ 2 „ „

10 „ „ „ 2 c.c. „ Berlin „ „ „ „ 2 „ „

courage the multiplication of any few anthrax bacilli that might still be present in the living state, and which would have escaped detection if the water had been directly plate-cultivated.

Gärtner (Gärtner-Tiemann's 'Untersuch. d. Wassers,' p. 588, Brunswick, 1889) again introduced anthrax bacilli into unsterilised drinking water at 12° C., and found that they had all disappeared on the sixth day (presumably culture tests only were employed).

Important experiments of a similar nature had previously been made by Kraus ('Archiv f. Hygiene,' vol. 6, p. 234) with unsterilised water at 10·5° C.; on introducing anthrax bacilli free from spores, he found them no longer recognisable by cultivation tests on the fourth day, as shown in the following table:—

Kraus's Experiments on the Vitality of Sporeless Anthrax in Unsterilised Waters.

Source of water.	Number of days after inoculation when examined.				
	1	2	4	8	130
	Number of anthrax bacilli found in 1 c.c. of water.				
Munich water supply........	1,150	900	0	0	0
Well water, Munich.........	1,050	1,000	0	0	0
" " "	1,180	850	0	0	0

A similar result was later obtained by Karlinski ('Archiv f. Hygiene,' 1889, pp. 113—127; 'Centralbl. f. Bakteriol.,' vol. 6, p. 139) in unsterilised drinking water at 8° C.; the anthrax bacilli free from spores were by him found to have disappeared on the third day.

Uffelmann ('Centralbl. f. Bakteriol.,' vol. 5, p. 89), on the other hand, introduced sporiferous anthrax into unsterilised drinking water at 12—20° C., and found that its vitality was preserved for upwards of three months.

The only experiments which have been made with British waters are those which were carried out by one of us (Percy Frankland, Society of Chem. Ind., 1887), in which sporiferous anthrax was introduced into sterile distilled water, sterile Grand Junction water (filtered Thames water), and sterile London sewage. In all cases the vitality of the anthrax was preserved for upwards of 61 days,

and in the sewage extensive multiplication of the organism actually took place, as will be seen from the following table (see p. 174).

If we endeavour to summarise the results obtained by these several investigators, the evidence would appear to point to the following conclusions:—

1. Spores of anthrax retain their vitality either in sterile or unsterilised waters of the most varied character for long periods of time, many months, at ordinary or low temperatures, whilst they are slowly destroyed if the waters are kept at 35° C.
2. The evidence concerning the sporeless anthrax bacilli is somewhat contradictory. Most observers agree that they are rapidly destroyed in a few days both in sterilised and unsterilised waters; Straus and Dubarry, however, using a more delicate method of cultivation, have found their vitality to be retained for a much longer period, viz., from 28 to 65 days. There can be little doubt that in these experiments spores were formed in the waters. The results of Wolffhügel and Riedel differ also from those of other observers, probably owing to their having added so much gelatine, inasmuch as they found the anthrax bacilli to undergo abundant multiplication in sterile waters at 16° and at 35° C., whilst rapid destruction took place if the same waters were maintained at 7—10° C.
3. As regards the power of anthrax to propagate in water, there is, with the exception of the last mentioned results of Wolffhügel and Riedel, no evidence that they undergo multiplication in ordinary potable waters even when sterilised; indeed, it has been clearly shown by one of us that no numerical increase takes place either in sterile distilled or sterile filtered Thames water (Grand Junction Company), whilst in sterile London sewage the numbers underwent very considerable multiplication. The power of propagation in sterile water at any rate is, therefore, dependent on its chemical composition. It should be mentioned that the multiplication observed by Wolffhügel and Riedel was not in potable water proper, but in the water of the River Panke at Berlin, which is, or was, practically diluted sewage, although they also obtained multiplication when this water was diluted with ten times its volume of distilled water. The whole question of multiplication, however, is doubtless to a large extent dependent on the vigour of the anthrax growths employed for experiment.
4. It is much to be regretted that so few investigators have made any experiments on the virulence of the anthrax organisms after their residence in waters under varied conditions; this is, after all, the chief point of practical importance, it is the point

Percy Frankland's Experiments on the Vitality of Sporiferous Anthrax in Water.

Sporiferous anthrax.	1st hour after infection.	2nd day.	5th day.	12th day.	21st day.	40th day.	61st day.
Distilled water—							
No. 1................	69	72	53	70	..	67 Contaminated with mould	110
No. 2................	65	65	53	80	..	89	36
No. 3................	106	67	87	63	..	88	100
Grand Junction water—							
No. 1................	340	230	67	81	..	75	100
No. 2................	530	175	44	95	..	67 Largely contaminated with small smooth-rimmed colony.	88
No. 3................	392	187	54	68	..	80	121
London sewage—							
No. 1................	753	39	82	Much multipled	2041	..	5543
No. 2................	108	240	114	498 Probably more.	547	..	290
No. 3................	289	145	129	Contaminated	..	..	..

which admits of the most ready determination, and it is quite unaccountable why in the experiments made on the Continent it should have been so frequently neglected.

Object and Nature of our Experiments.

One of our first objects has been to make ourselves acquainted with the nature of the waters, and especially that of the Thames, selected for investigation; and this not only as regards their chemical composition, but also as regards the nature and numbers of organisms normally found in the water. We have also made inquiries as to the changes the water undergoes on standing, and have acquired much interesting information regarding these points.

In pursuing the inquiry as to the vitality of anthrax in water, we have been guided by the following considerations. As already pointed out, we selected the *Bacillus anthracis* for the first series of our investigations on the vitality of pathogenic bacteria in water, because it constitutes almost the extreme term, so to speak, in the series of pathogenic organisms which are at present known. In the form of spores it presents one of the hardiest and most refractory examples of living organisms,* at any rate of the pathogenic kind, for amongst the non-pathogenic forms there are a number which excel it in this respect; whereas our information as regards the bacilli in water is most incomplete, though the whole practical interest as regards pathogenic organisms turns on their behaviour in the vegetative—spore-free—condition, and on whether they can multiply or develop spores in the water.

In introducing the anthrax bacilli and their spores into British waters of typical character, we have endeavoured to ascertain whether their fate is affected (*a*) by differences of temperature such as occur in the natural course of events, (*b*) by the other bacteria present in the water; and, in order to ascertain this point, we have employed the waters in question in their natural state, unsterilised; also sterilised by heat (steam) in the ordinary way; and, thirdly, sterilised without the application of heat by filtration through unglazed porcelain.

We have also endeavoured to ascertain whether the sporiferous anthrax bacilli are differently affected according as the water in which they are resident is kept in darkness, placed in diffused light, or exposed to direct sunshine.

* Pasteur showed ('Compt. Rend.,' 1877, vol. 85, p. 99) that they remain alive for some time in absolute alcohol, and for twenty-one days exposed to a pressure of 10 atmos. of pure oxygen. Koch has shown ('Mitth. a. d. Kaiserl. Ges. Amt.,' I, p. 32) what extreme temperatures they will endure, and Klein declares that ten minutes' boiling cannot be relied on. We have already given the literature showing that the spores remain for long periods alive in water, and it is well known they can be kept intact for months on silk threads in the dark.

Finally, we have not merely confined ourselves to the ordinary cultivation tests for ascertaining the vitality or otherwise of the anthrax organism under these several conditions; but we have also submitted the virulence of the infected waters under investigation to the direct test of inoculation into animals.

Inasmuch as the investigation of the above points has been conducted by each of us to a certain extent independently of the other, it has been proposed to record our experiments separately also, as in this manner the course of the two investigations is most easily followed, and the results obtained most readily surveyed. In the following pages, therefore, will be found a separate account of the independent inquiry pursued by each of us, the report closing with a number of conclusions which we have together drawn from the experimental material collected by both.

PART I.

"Experiments on the Vitality and Virulence of Sporiferous Anthrax in Potable Waters." By Professor PERCY FRANKLAND, Ph.D., F.R.S., assisted by J. R. APPLEYARD, F.C.S.

The first water selected for experiment was that of the River Thames, which may be taken as a type of a calcareous surface water draining from cultivated land, but receiving only such a moderate proportion of organic impurity as to leave it in a condition that, judged by the ordinary standards of taste, smell, and appearance to the eye, it is suitable for drinking purposes. This is in fact the water which has for years been supplied to the larger part of the metropolis, and is, therefore, in some respects the most interesting water, from a hygienic point of view, in the United Kingdom.

The second water experimented with is that of Loch Katrine as supplied to Glasgow, which again is typical of those upland surface waters, collected from almost entirely uninhabited areas, which have been so largely utilised during the past 30 years for the supply of the great manufacturing districts of the north. These waters, and notably that of Loch Katrine, are characterised by their great softness and almost entire freedom from mineral matters, whilst the organic constituents are almost wholly of vegetable origin, and thus differ more qualitatively than quantitatively from those present in waters such as that of the Thames.

Experiments with Thames Water.

In order to render the experiments as comparable as possible they have all been made on one and the same sample of Thames water, which was collected personally by my colleague, Professor Marshall

Summary of the Monthly Reports on the Bacteriological Condition of the London Water-Supply presented to the Local Government Board by Percy F. Frankland in 1886.

Number of Colonies obtained from 1 c.c. of Water by Gelatine-plate Cultivation.

Name of supply.	Jan.	Feb.	March.	April.	May.	June.	July.	August.	Sept.	Oct.	Nov.	Dec.	Average for year.
THAMES.													
Thames water, unfiltered (Hampton)	45,000	15,800	11,415	12,250	4,800	8,300	3,000	6,100	8,400	8,600	56,000	63,000	..
Chelsea	159	305	299	94	59	60	59	303	87	34	65	222	..
West Middlesex	180	80	175	47	19	145	45	25	27	22	47	2,000	..
Southwark	2,270	284	1,562	77	29	94	380	60	49	61	321	1,100	..
Grand Junction	4,894	208	379	115	51	17	14	12	17	77	80	1,700	..
Lambeth	2,587	265	287	209	136	129	155	1,415	59	45	108	305	..
Reduction per cent...	95·6	98·6	95·3	99·1	98·8	98·9	95·6	94·0	99·4	99·4	99·8	98·3	97·6
LEE.													
Lee water, unfiltered (Chingford)	39,300	20,600	9,025	7,300	2,950	4,700	5,400	4,300	3,700	6,400	12,700	121,000	..
New River	363	74	95	60	22	53	46	55	17	10	32	400	..
East London........	224	252	533	269	143	445	134	243	165	97	248	280	..
Reduction per cent.*	99·4	98·8	94·1	96·3	95·2	90·5	97·5	94·3	95·5	98·5	98·0	99·8	96·5
DEEP WELLS (Kent Company).													
Bath Well	..	..	..	..	..	..	..	..	..	..	12	10	..
Garden Well	..	..	..		..	..	..	..	..	..	..	..	..
New Well........ {	..	5†	44†	7†	8†	4†	12†	9†	5†	3†	..	..	..
	..	..	..	..	..	..	..	..	..	160†	..	11	..
Supply	43	149	38	47	101	39	48	13	25	{283 405}	196	66	..

* These reductions apply only to the East London supply.

† In all cases marked with a dagger the name of the particular well was not mentioned.

Summary of the Monthly Reports on the Bacteriological Condition of the London Water-Supply presented to the Local Government Board by Percy F. Frankland in 1887.

Number of Colonies obtained from 1 c.c. of Water by Gelatine-plate Cultivation.

Name of supply.	Jan.	Feb.	March.	April.	May.	June.	July.	August.	Sept.	Oct.	Nov.	Dec.	Average for year.
THAMES.													
Thames water, unfiltered (Hampton)	30,800	6,700	30,900	52,100	2,100	2,200	2,500	7,200	16,700	6,700	81,000	19,000	..
Chelsea	5,300	81	171	55	49	190	106	44	73	64	187	86	..
West Middlesex	258	27	96	110	32	123	40	87	82	28	53	113	..
Southwark	4,900	428	1,325	360	61	196	119	70	84	130	152	133	..
Grand Junction	7,500	612	443	109	48	103	35	78	15	80	55	80	..
Lambeth	1,200	188	884	103	53	521	108	733	85	96	1,120	198	..
Reduction per cent.	87·6	96·0	98·1	99·7	97·7	89·7	96·7	97·2	99·6	98·8	99·6	99·4	96·7
LEE.													
Lee water, unfiltered (Chingford)	37,700	7,900	24,000	1,330	2,200	12,200	12,300	5,300	9,200	7,600	27,000	11,000	..
New River	508	72	133	38	16	31	33	15	23	25	41	39	..
East London	6,700	100	182	127	105	1,200	194	104	169	148	190	456	..
Reduction per cent.*	82·2	98·7	99·2	90·5	95·2	90·2	98·4	98·0	98·2	98·1	99·3	95·9	95·3
DEEP WELLS (Kent Company).													
Bath Well	9	19	80	26	27	12	14	5	5	7	3	6	..
Garden Well	48	20	4	4	—	24	18	—	8	..	5	12	..
New Well	12	10	5	12	20	14	8	59	27	30	65	67	..
Supply	82	75	140	163	50	26	44	116	115	357	40	68	..

* These reductions apply only to the East London supply.

Summary of the Monthly Reports on the Bacteriological Condition of the London Water-Supply presented to the Local Government Board by Percy F. Frankland in 1888.

Number of Colonies obtained from 1 c.c. of Water by Gelatine-plate Cultivation.

Name of supply.	Jan.	Feb.	March.	April.	May.	June.	July.	August.	Sept.	Oct.	Nov.	Dec.	Average for year.
THAMES.													
Thames water, unfiltered (Hampton)	92,000	40,000	66,000	13,000	1,900	3,500	1,070	3,000	1,740	1,130	11,700	10,600	..
Chelsea	127	152	54	38	43	63	37	32	36	14	82	71	..
West Middlesex	60	146	408	158	71	56	27	11	26	33	31	16	..
Southwark	177	766	742	47	47	24	35	27	106	35	167	136	..
Grand Junction	90	349	617	56	77	40	15	4	20	16	25	208	..
Lambeth	189	820	321	157	64	140	55	33	92	27	126	151	..
Reduction per cent.	99·9	98·9	99·4	99·3	96·8	98·1	96·8	99·3	96·8	97·8	99·3	98·9	98·4
LEE.													
Lee water, unfiltered (Chingford)	31,000	26,000	63,000	84,000	1,124	7,000	2,190	2,000	1,670	2,310	57,500	4,400	..
New River	27	90	169	77	37	60	11	13	—	15	70	91	..
East London	2,038	780	359	193	209	266	253	57	64	63	49	141	..
Reduction per cent.*	93·4	97·0	99·4	99·8	81·4	96·2	88·4	97·2	96·2	97·3	99·9	96·8	95·3
DEEP WELLS (Kent Company).													
Bath Well	6	47	6	33	7	17	8	—	8	4	34	—	..
Garden Well	5	19	8	4	27	71	5	—	10	9	18	—	..
New Well	12	4	5	7	8	20	4	3	—	96	19	—	..
Supply	55	81	15	69	139	219	32	42	52	55	54	63	..

* These reductions apply only to the East London supply.

Ward, on March 8, 1892, at a point more than a mile above Staines, and sufficiently distant from the Windsor district to render it very improbable that any direct contamination thence need be feared; as a matter of fact, the analyses show that this water was by no means rich either in bacteria or organic matter, and, for an open river, was remarkably pure (see p. 182).

As this sample was not received by me until some days after its collection, the number of micro-organisms which I found in it does not afford any insight into the bacterial condition of the river at the time, but on this subject I have already collected a large amount of information in the course of the regular monthly examinations of the London water supply which I made for the Local Government Board during the three years 1886, 1887, and 1888. The results of these examinations, which are recorded in the preceding three tables, clearly indicate (1) the seasonal variations which the number of bacteria in the unfiltered waters of the Rivers Thames and Lee undergoes; (2) the great reduction in these numbers which is effected by the storage and sand-filtration to which these waters are subjected at the waterworks before distribution; (3) the very small number of bacteria present in the deep-well water of the Kent Company.

Two entirely independent series of experiments have been made with this sample, the difference between the two series being in the number of anthrax organisms which were introduced into the water. Thus in the First Series, a comparatively small number of anthrax bacilli were put into the water, whilst in the Second Series the number introduced was very much larger. There was this further difference between the two series of experiments that the *Bacillus anthracis* employed had a totally different origin in the two cases. The use of the organism from two distinct sources in the way indicated is in my opinion of great importance as eliminating the possibility of any special and exceptional characters having become impressed on the particular cultivation employed.

Experiments with Thames Water (First Series).

We will direct our attention in the first instance to what we have called the "First Series" of experiments, in which the sporiferous *Bacillus anthracis* was introduced in comparatively small numbers only into the water.

This First Series of experiments includes four sub-series, in each of which the Thames water was employed in a different condition. Thus—

(1.) Experiments made with the Thames water in its natural state.

(2.) Experiments made with the Thames water after removing the coarser suspended particles by filtration through Swedish filter

paper. This was done, as it was quite conceivable that the presence of comparatively large suspended particles should exercise a marked influence on the behaviour of the anthrax introduced, whilst this filtration would not remove more than a portion of the bacteria already present in the water in its natural state. It was, moreover, especially desirable to ascertain whether the removal of these coarser suspended particles would influence the result, as in the following sub-series (3) all suspended particles, including the water bacteria themselves, were removed prior to the introduction of anthrax.

(3.) Experiments with the Thames water after removing all suspended particles, including bacteria, by filtration through porous porcelain (Chamberland filter), or, in other words, Thames water sterilised without the agency of heat.

(4.) Experiments with the Thames water after filtration through Swedish paper as in sub-series (2), and subsequent sterilisation with steam.

Chemical Composition of the Thames Water employed.—The water was submitted to analysis, (a) *in its natural condition*, (b) *after filtration through Swedish paper*, and (c) *after filtration through porous porcelain*, with the following results:—

Results of Analysis expressed in Parts per 100,000.

	(*a*).	(*b*).	(*c*.)
Total solid residue (dried at 100° C.)	35·20	33·60	33·60
Organic carbon	0·207	0·212	0·189
,, nitrogen	0·023	0·039	0·021
Ammonia (free)	0·004	0·003	0·007
,, (albuminoïd)	0·016	0·010	0·014
Oxygen consumed by organic matter, as measured by the reduction of permanganate acting for three hours in the cold	0·076	0·054	0·064
Nitrogen as nitrates and nitrites	0·230	0·272	0·229
Total combined nitrogen	0·256	0·313	0·256
Chlorine	1·6	1·65	1·7
Temporary hardness	17·3	17·1	16·5
Permanent ,,	5·1	5·3	5·9
Total ,,	22·4	22·4	22·4
Remarks	very turbid	clear	clear

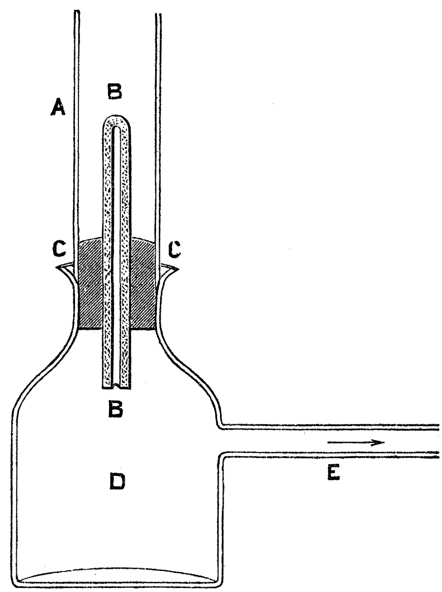
These results show that the sample contained only a moderate amount of organic matter, and was representative of the river when in its purest condition. They also show that neither the filtration

through paper nor through porcelain exerts any material effect on the chemical composition of the water, such differences as appear being almost within the limits of experimental error, especially when it is borne in mind that (*a*), (*b*), and (*c*) were taken from different bottles.

Filtration of the Water through Porcelain.—The sterilisation of the water without application of heat was conveniently effected in the following manner:—

A glass cylinder (A) open at both ends is tightly fitted to an india-rubber stopper (CC), which is also fitted into the strong

FIG. 1.



Porcelain Filter.

glass bottle (D), a porous cylinder of biscuit-porcelain of the construction shown in section in (BB) also passes through the same india-rubber stopper. The bottle (D) has a lateral tubulure (E) which is plugged with sterile cotton-wool. The whole of this apparatus is sterilised in position by placing it bodily in the steam-steriliser for several hours on three successive days, after which it is ready for use. The water to be filtered is poured into the glass cylinder (A), and the tubulure (E) is connected by means of pressure tubing with a water-pump. On thus reducing the pressure inside (D), the water in (A) is forced by atmospheric pressure through the porous cylinder (BB). In order to remove the filtered water

from (D), the cotton-wool plug is extracted from (E), the latter tube is carefully heated with a Bunsen flame to destroy any organisms that may be resting on its open extremity, and by inclining (D) the water can be made to flow out into the sterilised vessels placed for its reception without undergoing any contamination.

Infection of the Water with Anthrax.—In this first series the anthrax bacilli were taken from an agar-agar cultivation of about three weeks age, in which it was known by previous microscopic examination that spores were abundantly present.

The surface of the agar-agar was scraped with a sterile platinum loop, care being taken to remove the growth with as little as possible of the culture material. Five loops full in all were taken and transferred to a small sterile stoppered bottle containing about 50 c.c. of Thames water which had been steam-sterilised. The contents were then violently agitated for some fifteen minutes in order to break up the conglomerations of bacilli and spores, and effect as uniform a distribution as possible. This may be termed the "first attenuation."

From this first attenuation four portions of 2 c.c. each were removed with sterile pipettes and introduced respectively into four large flasks, each containing about 2 litres of the waters for experiment, viz.:—

(*a.*) Thames water, in natural state.
(*b.*) Ditto after filtration through Swedish paper.
(*c.*) Ditto after filtration through porcelain.
(*d.*) Ditto, after sterilisation by steam.

The waters thus infected were well shaken in the large flasks containing them so as to ensure complete mixture, and the contents of each flask was then distributed in a number of small sterilised conical flasks plugged with sterile cotton wool. In the case of each of these infected waters some of the small conical flasks were placed in an incubator maintained at 18—20° C., the summer temperature of surface waters, whilst others were put into a refrigerator in which a temperature of 6—10° C. was preserved.

The distribution and arrangement will be readily apparent from the following tabular statement:—

Thames Water in Natural State.

Un-infected	2 flasks in incubator.	Infected	4 flasks in incubator.
	,, refrigerator.		3 ,, refrigerator.

Thames Water after Filtration through Swedish Paper.

Un-infected	2 flasks in incubator.	Infected	5 flasks in incubator.
	,, refrigerator.		,, refrigerator.

Thames Water after Filtration through Porcelain.

Un-infected	2 flasks in incubator.	Infected	5 flasks in incubator.
	,, refrigerator.		,, refrigerator.

Thames Water after Sterilisation by Steam.

Un-infected	2 flasks in incubator.	Infected	5 flasks in incubator.
	,, refrigerator.		,, refrigerator.

Thus, there were in all 16 uninfected and 37 infected flasks employed.

Note.—Throughout the subsequent account of the series, all flasks placed in the incubator are designated thus: "1 I," "2 I," "3 I," &c., whilst flasks placed in the refrigerator are distinguished as "1 R," "2 R," "3 R," &c.

Examination of the Waters for the Presence of Anthrax.—The bacteriological examination for the presence of anthrax was in general made by the ordinary process of gelatine plate cultivation. This method of identifying the presence of anthrax is attended with but little difficulty if no other organisms are simultaneously present, as in the case of the waters sterilised by steam, and by filtration through porcelain. The anthrax colonies develop with such facility in the gelatine medium, and are of such a characteristic appearance even to the naked eye, but especially when seen through a low power of the microscope, that no doubt can be entertained as to their identity. On the other hand, the very greatest difficulty attends, as will be seen, the recognition of anthrax in the presence of the ordinary water bacteria, partly because the colonies of some of the latter grow much more quickly than those of anthrax, but especially because many of these water bacteria cause such rapid liquefaction of the gelatine that the greater part, or even the whole, of the film may be destroyed before the anthrax colonies have had time to become visible. In order to overcome this difficulty, I have devised a method of destroying nearly the whole of these liquefying bacteria without injuring more than a part of the anthrax spores, and thus rendering possible the development and recognition of the colonies from the latter, even when they are present in water along with vast multitudes of the ordinary water bacteria. The nature of this special method will be described later. The gelatine plates were invariably incubated at a temperature of 18—20° C., and in order to give every opportunity for the anthrax colonies to make their appearance, the incubation was carried on as long as possible. On this account the numerical estimation of the other colonies was made a subsidiary matter, and in consequence of the extensive liquefaction which had often taken place, the accuracy of the numbers found has often been interfered with; in fact, discrepancies in the number of colonies found on dupli-

Tabular Description of Bacteria isolated from the Waters of the Rivers Thames and Lee, and from Deep Wells in the Chalk (Grace and Percy Frankland, 'Zeitschrift für Hygiene,' vol. 6, 1889).

Name of micro-organisms and where found.	Microscopic appearance.	Appearance in gelatine-plate culture.	Gelatine-tube cultivations.	Agar-agar cultivations.	Broth cultivations.	Potato cultivations.	Growth and action in nitrate solution.
No. 1.—*Bacillus arborescens.* Filtered river water of Thames and Lee.	Slender bacillus with rounded ends, about 2·5 μ long and 0·5 μ broad. Hangs together in twos and threes, but in broth cultures forms long wavy threads. No spore formation observed. Is capable of vibratory movement only.	Under a low power (× 100 diameters) is seen to form a thin axial stem, from both ends of which root-like branches extend, which gradually assume the appearance of a wheat-sheaf. Slow liquefaction of the gelatine takes place, and near the colony the surface of the gelatine exhibits beautiful iridescent colours.	Slowly liquefies the gelatine, producing a yellow deposit.	Produces a dirty orange coloured pigment, and grows slowly.	Renders the liquid turbid, and produces yellow deposit. No pellicle forms on the surface.	Produces a luxuriant and deep orange growth.	No visible growth takes place; neither is any reduction of the nitrate to nitrite effected.
No. 2.—*Bacillus aquatilis.* Deep-well water obtained from the chalk.	Very similar in appearance to No. 1, and forms also wavy threads, sometimes as long as 17 μ and more. No spores observed. Vibratory movement only.	In the depth the colonies at first appear smooth rimmed, but the contour gradually becomes more and more irregular. On reaching the surface slow liquefaction of the gelatine commences, and convoluted bands of threads extend from the centre to the periphery.	Grows extremely slowly; forms a slightly yellow expansion on the surface, but hardly any growth appears in the depth. Later slight liquefaction takes place.	Produces a small shining yellow growth.	Renders it turbid, and produces a whitish deposit. No pellicle is formed.	Hardly any growth at all.	Grows abundantly, but fails to convert the nitrate to nitrite.
No. 3.—*Bacillus liquidus.* Rivers Thames and Lee.	Short thick bacillus with rounded ends; occurs usually in pairs, the dimensions of which are very variable (from 1·5 to 3·5 μ). No spores were found. It is very motile.	In the depths the colonies are smooth-rimmed; later they become jagged. It grows rapidly and causes extensive liquefaction of the gelatine, producing large circular depressions with almost clear contents.	Grows rapidly, forming a funnel-shaped depression, which is filled with turbid liquid. A thin pellicle forms later on the surface.	Produces a small shining expansion, and grows luxuriantly.	Renders it turbid, producing an abundant deposit, also a pellicle on the surface.	Produces a thick flesh coloured pigment.	Powerfully reduces the nitrate to nitrite.

No. 4.—*Bacillus vermicularis.* River Lee.	Large bacillus with rounded ends, in length about 2 to to 3 μ, and about 1 μ broad. Forms extensive vermiform threads. Produces oval spores about 1·5 μ long, and 1 μ broad. It is not motile.	The colonies in the depth are irregular in contour. This irregularity increases as the liquefaction commences, and the colony approaches the surface. The periphery is seen to consist of closely packed wavy bands of bacilli, whilst the centre of the colony looks irregular and wrinkled.	Forms a moist shining grey expansion, whilst in the depth the path of the needle is indicated by a slight sword-like growth. Slow liquefaction of the gelatine takes place.	Produces a smooth shining grayish pigment.	The liquid remains clear, whilst a considerable white flocculent deposit is formed.	Produces a thick irregular flesh coloured pigment.	Powerfully reduces the nitrate to nitrite.
No. 5.—*Bacillus nubilus.* River Thames.	Slender bacillus, about 3 μ long and 0·3 μ broad. Forms long wavy threads in broth cultures. No spores were found. The isolated bacilli exhibit violent rotatory movements, but the threads are quite stationary.	Forms cloudy undefined patches, which under the microscope are seen to consist of a thick and tangled mass of bacillar threads. Rapid liquefaction of the gelatine takes place.	The surface is liquefied, bnt all along the path of the needle a series of horizontal circular plates arise, having a delicate cloud-like appearance. Later the whole of the gelatine becomes liquid.	Produces a thin opalescent blue-violet expansion, the edges of which exhibit later a distinct violet fluorescence.	Renders it turbid and produces a dirty white deposit, whilst the surface becomes covered with a thin pellicle.	Produces a delicate and slightly yellow growth which is barely visible.	Reduces a very small proportion of the nitrate to nitrite.
No. 6.—*Bacillus ramosus.* Frequently found in Rivers Thames and Lee, but never in deep-well water.	Much resembles *B. subtilis.* The individual bacilli are about 7 μ long and 1·7 μ broad, the ends being distinctly rounded. It gives rise to long threads, also spores. Is capable of only slight oscillatory movement.	The colonies are seen to consist of cloudy centres with tangled root-like branches extending in every direction. Later liquefaction of the gelatine takes place.	The whole contents of the tube become impregnated with fluffy ramifications. Later liquefaction takes place, and a tough pellicle forms on the surface.	Grows rapidly over the whole surface, whilst in the depth the characteristic "branching" is again visible.	Forms a light flocculent deposit, and produces later a tough and wrinkled pellicle on the surface.	Produces a dry and uniform expansion, which is almost quite white.	Powerfully reduces the nitrate to nitrite.
No. 7.—*Bacillus aurantiacus.* Deep-well water.	Short fat bacillus of very variable dimensions. It grows in pairs, and also forms long threads. The short bacilli are about 1·7 μ long, and nearly half as wide as long. No spores were observed. The individual bacilli are motile.	Produces bright orange pinheads. Under the microscope the depth colonies are seen to be smooth-rimmed. No liquefaction of the gelatine takes place, and its growth is slow.	A shining orange coloured expansion forms on the surface, whilst hardly any growth is visible in the depth.	Forms a bright orange expansion, which does not extend much beyond the point of inoculation.	The liquid remains clear, whilst a slightly orange coloured deposit is produced. A thin pellicle forms on the surface, which exhibits here and there bright spots of orange colour.	Produces a thick and magnificently brilliant red-orange pigment which is however restricted to the point of inoculation.	Reduces the nitrate to nitrite only very slightly.

Tabular Description of Bacteria isolated from the Waters of the Rivers Thames and Lee, and from Deep Wells in the Chalk (Grace and Percy Frankland)—*continued*.

Name of micro-organisms and where found.	Microscopic appearance.	Appearance in gelatine-plate culture.	Gelatine-tube cultivations.	Agar agar cultivations.	Broth cultivations.	Potato cultivations.	Growth and action in nitrate solution.
No. 8.—*Bacillus viscosus.* Found very frequently in unfiltered river water of the Thames and Lee, also occasionally in the same water after filtration, whilst it is only rarely found in deep-well water.	Short bacillus with rounded ends, from 1·5 to 2 μ long, about three or four times as long as broad. Occurs usually in pairs. No spores observed. It is exceedingly motile.	In the depth the colonies appear smooth-rimmed; later, when liquefaction commences, the periphery exhibits fine hair-like extensions. The gelatine is rapidly liquefied, and each colony is surrounded by a green fluorescent zone.	Causes rapid liquefaction of the gelatine, producing green fluorescence throughout the contents of the tube, which becomes excessively viscid.	The whole surface rapidly assumes a green appearance, and a smooth green-white expansion is produced.	Renders the liquid very turbid and viscid. Later a thin green-white pellicle is formed.	Produces a moist and shining chocolate coloured expansion, which extends over the whole surface.	No reduction of the nitrate takes place.
No. 9.—*Bacillus violaceus.* Originally found in River Spree water. but found by us also in the Rivers Thames and Lee, also in deep-well water from the chalk.	Short bacillus, varying in size, about 1·7 μ in length and 0·8 μ in width. It generally occurs in pairs. When grown on agar it assumes a far more slender appearance, and also gives rise to short threads. Spore formation was observed. It is motile, but restricted chiefly to vibratory and rotatory movements.	In the depth the colonies appear irregular in contour, which increases with the age of the colony. It forms a circular depression in the gelatine, and later the characteristic violet pigment makes its appearance. Liquefaction does not take place rapidly.	Liquefaction takes place in the form of a funnel, the liquid becomes turbid and at the bottom of the funnel the violet pigment collects.	Forms a beautiful deep violet coloured expansion, which spreads over the whole surface.	The liquid is rendered slightly turbid, and later on a violet deposit is produced.	It is unable to grow on potatoes.	Powerfully reduces nitrates to nitrites.

cate plates cannot fail to take place if they are preserved until wide-spreading liquefaction of the gelatine has occurred.

1. *Bacteriological Examination of the Thames Water previous to Infection with Anthrax.*

From Table I it will be seen that the unfiltered water contained a large number of micro-organisms, which was, however, much reduced by the simple process of filtration through Swedish paper. The colonies obtained showed the micro-organisms to be of numerous different kinds, many of them being easily recognisable as belonging to the forms which have already been described and figured by me three years ago (Grace C. and Percy F. Frankland, 'Zeitsch. f. Hygiene,' vol. 6, 1889) as occurring in Thames water, and a brief *résumé* of which is given in the following table. Owing to a large number of the colonies causing liquefaction of the gelatine, their numerical estimation is much interfered with, and in order to some extent obviate this difficulty, it will be seen that plates were not only poured with the undiluted waters, but also with the waters after large dilution (50 times their volumes) with sterilised water, so as to obtain a smaller number of colonies on the plate. This expedient has always been resorted to in cases where an inconveniently large number of colonies was to be expected; in all cases, however, the results are calculated to the number of colonies obtained from 1 c.c. of the water in the undiluted state.

Table I also shows that the filtration through porcelain, as well as the steaming, were effectual in sterilising the water, the number of colonies obtained in these cases being no greater than would appear on blank plates.

The unfiltered Thames water, and that which had been passed through Swedish paper, were again examined on October 26, 1892, or more than seven months after the experiments were commenced; in both cases the flasks which had been kept in the incubator contained more organisms than those which had been in the refrigerator, but in all cases the numbers were comparatively small, and in the unfiltered water had fallen much below what they were at the beginning.

Table I.—Uninfected Thames Water. First Series of Experiments, commenced March 18, 1892.

Dates on which plates were poured.	Water used.	Number of plate.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies from 1 c.c. of water. Total number.	
				c.c.		
18.3.92	Unfiltered	17	5	$\frac{1}{4}$	15,376	
		18	5	$\frac{1}{6}$	17,163	
		19	7	$\frac{1}{60}$	38,700	
		20	7	$\frac{1}{200}$	20,400	
18.3.92	Paper filtered	13	4	$\frac{5}{12}$	2,952	
		14	7	$\frac{1}{12}$	3,256	
		15	7	$\frac{1}{50}$	2,600	
		16	7	$\frac{1}{100}$	1,100	
18.3.92	Porcelain filtered	21	8	1	2	
		22	8	$\frac{1}{2}$	6	
18.3.92	Steamed	23	8	1	5	
		24	8	$\frac{1}{2}$	0	
26.10.92	Unfiltered.					
	1 I*	510	3	$\frac{5}{11}$	4,312	
	1 I	511	3	$\frac{1}{11}$	4,851	
	1 I	514	4	$\frac{1}{60}$	4,950	
	1 I	515	4	$\frac{1}{120}$	6,100	
	1 R	512	3	$\frac{5}{12}$	1,622	
	1 R	513	3	$\frac{1}{12}$	1,368	
	1 R	516	4	$\frac{1}{60}$	1,950	
	1 R	517	4	$\frac{1}{120}$	1,900	

26.10.92	Paper filtered. 1 I	518	3	$\frac{1}{3}$	1,986	
	1 I	519	3	$\frac{1}{15}$	3,150	
	1 I	522	3	$\frac{1}{60}$	4,400	
	1 I	523	3	$\frac{1}{120}$	4,300	
	1 R	520	3	$\frac{5}{14}$	935	
	1 R	521	3	$\frac{1}{14}$	1,090	
	1 R	524	3	$\frac{1}{60}$	1,600	
	1 R	525	3	$\frac{1}{120}$	1,450	

* For explanation of the system of naming the flasks containing the experimental waters, see Note, p. 185.

2. *Bacteriological Examination of the Unsterilised Unfiltered Thames Water (First Series) after Infection with Anthrax.*

Table II brings out a number of points. In the first place the extreme difficulty of discovering, by ordinary plate cultivation, a particular micro-organism when present in only small numbers alongside of vast multitudes of other forms, is particularly noteworthy. Special reference was made to this difficulty in our First Report, and it is most strikingly brought out in these experiments. From the experiments made with the steamed and porcelain-filtered Thames water, to be described below, we know that the number of anthrax organisms in this unfiltered Thames water must have amounted to at least 30—40 per cubic centimetre, yet only in one out of the numerous plate cultivations made with this water was anthrax discovered, and then only a single colony was found on a plate poured on the day that the infection with anthrax was made.

The principal obstacles to such discovery are two: firstly, in consequence of the extremely large number of micro-organisms present in the water, it is only possible to take a very small volume (not more than, say, $\frac{3}{10}$ c.c.) for cultivation, in which, therefore, the chance of anthrax organisms (if introduced in such small numbers as in the present series of experiments) being present is very remote; whilst, secondly, owing to the rapid liquefaction of the gelatine caused by many of these water bacteria, it is not possible generally to incubate the plates for a sufficient length of time to admit of the proper development of the anthrax colonies; this is more particularly the case when, as here, the anthrax is present in the form of spores, which take time to germinate.

In order to obviate these difficulties attending the discovery of anthrax in the presence of large numbers of water bacteria causing liquefaction, I have tried a number of special devices, of which I need, however, only describe the one which proved the most useful.

Previous experience had shown me that a large proportion of the organisms present in water, and more especially those causing liquefaction of the gelatine, are very sensitive to a temperature even considerably below that of boiling water, whilst the spores of anthrax in their normal state will withstand such temperatures for a considerable length of time.

In order to turn these properties to practical account, portions (1 c.c. or 3 c.c.) of the anthrax-infected Thames water under consideration were mixed with a little sterile broth (1 c.c.), and heated for periods of two or five minutes to 50° C., to 70° C., and to 90° C., after which treatment they were submitted to ordinary plate cultivation. The first of these experiments was made on March 31, 1892, and is recorded in the above Table II. The infected Thames

water at this time contained upwards of 100,000 water bacteria in 1 c.c., yet after heating as above for five minutes to 50° C., only from 35 to 39 colonies per cubic centimetre made their appearance, and amongst these several were easily recognisable as those of anthrax. Again, on the same day, other portions of the same water were heated to 70° C. for two minutes, after which only from 10 to 30 colonies per cubic centimetre made their appearance, amongst which from 4 to 10 were recognisable as anthrax. Other portions of the same water were heated on the same day to 90° C. for two minutes, with the result that only from 7 to 10 colonies per cubic centimetre appeared, of which from 3 to 6 were found to be anthrax.

Thus by this simple method comparatively large volumes (up to 3 c.c. have been used, but there is no reason why even larger quantities should not, if necessary, be employed) of water swarming with water bacteria can be operated on and sifted, so to speak, for anthrax.

From Table II it will be seen that this method was repeatedly employed on the anthrax-infected unfiltered Thames water in question, in most cases the temperature of 70° C. for two minutes being resorted to.

Employing this method it will be seen from the table that *it became more and more difficult to discover anthrax in the water, although even after nearly four months anthrax could still be just traced both in the water, which had remained at summer* (18—20° C.) *temperature in the incubator, as well as in that preserved at the winter temperature* (6—10° C.) *of the refrigerator.*

It now became of interest to ascertain whether the water in which anthrax could just be barely traced by cultivation contained that anthrax in a virulent state. In consequence of the delay which occurred in my obtaining the necessary licence to perform these experiments, I was not able to attack this problem until October 7, 1892, or nearly seven months after the water was infected with anthrax.

Animal Experiment No. 1.—On October 7, 1892, 1 c.c. of water from "Flask 3 I, unfiltered Thames water infected with anthrax, March 18, 1892," was subcutaneously injected into a white mouse. The mouse did not succumb to anthrax, but is still living, 32 days after the operation.

Animal Experiment No. 2.—On the same day, October 7, 1892, 1 c.c. of water from "Flask 3 R, unfiltered Thames water, infected with anthrax, March 18, 1892," was subcutaneously injected into a white mouse. This mouse lived for 18 days 20½ hours after the operation, and, of course, did not succumb to anthrax; no bacilli could be found in the spleen, nor by cultivation in gelatine.

From these experiments it is obvious that on October 7, 1892, or nearly seven months after infecting this unfiltered Thames water

with sporiferous anthrax bacilli, the latter had been so much reduced in number, that a whole cubic centimetre of the water, irrespectively of whether it had been kept at the winter temperature of the refrigerator, or at the summer temperature of the incubator, was unable to cause the death of a mouse, the most sensitive of animals to anthrax.

It was in the next instance necessary to ascertain whether the sporiferous anthrax had actually perished outright in this water, or whether it was still present in a living state only in very small numbers. To determine this point, the following plan was adopted:—The two flasks referred to above, 3 I and 3 R, were each treated with 5 c. c. of sterile broth on October 15, 1892, and placed in an incubator at 37° C., so as to encourage the growth and multiplication of any anthrax bacilli or spores that might still be living in them. With these nourished waters, so to speak, then, the following two experiments were made:—

Animal Experiment No. 16.—On October 18, 1892, 0·4 c.c. from the Flask 3 I, to which broth had been added on October 15, 1892, was subcutaneously injected into a white mouse. The mouse died within 1 day 19 hours, and, although no bacilli could be microscopically detected in the spleen, a gelatine tube inoculated from the spleen developed a typical growth, and in this the characteristic anthrax bacilli were subsequently found, thus leaving no doubt that the mouse had died of anthrax.

Animal Experiment No. 24.—On October 29, 1892, 0·5 c.c. from the Flask 3 R, to which broth had been added on October 15, 1892, was subcutaneously injected into a white mouse. The mouse died within 2 days 18 hours; the spleen was found to be full of anthrax bacilli, and gelatine cultivations prepared from the spleen yielded the characteristic growths, thus leaving no doubt that the animal succumbed to anthrax.

These two experiments clearly demonstrate that the sporiferous anthrax bacilli had not become actually extinct in this unfiltered Thames water (either at winter or summer temperature), but had only undergone great numerical decline, for on adding broth to the waters these straggling forms multiplied sufficiently to cause the death of the mice which received subcutaneous injections of them.

Of subsidiary interest in Table II is the insight furnished into the behaviour of the ordinary water bacteria at the temperatures in question. These numbered about 10,000 per cubic centimetre at the outset,* whilst after 4 days (March 22, 1892) about

* This does not refer to the number present in the water at the time of its collection from the Thames, but at the time of its infection with anthrax several days later. The sample was collected on March 8, and the infection was made on the 18th of the same month.

Table II.—Anthrax in Thames Water. First Series of Experiments. Unfiltered Thames Water, Infected with Anthrax, March 18, 1892.

Dates on which plates were poured.	Number of plate.	Particular flask employed. *	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies from 1 c.c. of water.		Remarks.
					Total number.	Number of anthrax.	
				c.c.			
18.3.92	9	..	4	$\frac{3}{10}$	8,540	0	The Thames water collected on 8.3.92, remained in stoppered bottles at 10° C. until 18.3.92, when these experiments were commenced.
	10	..	7	$\frac{1}{10}$	Too much liquefied	10	
	11	..	7	$\frac{1}{120}$	12,600	0	
	12	..	7	$\frac{1}{300}$	13,200	0	
22.3.92	34	3 I	3	$\frac{5}{12}$	Too much liquefied	0	
	35	3 I	3	$\frac{1}{12}$	49,800	0	
	42	3 I	4	$\frac{1}{55}$	113,850	0	
	43	3 I	4	$\frac{3}{550}$	91,850	0	
	36	3 R	3	$\frac{5}{12}$	Too much liquefied	0	
	37	3 R	3	$\frac{1}{12}$	127,680	0	
	44	3 R	4	$\frac{1}{55}$	143,550	0	
	45	3 R	4	$\frac{3}{550}$	141,900	0	
31.3.92	79	2 R	4	3	35	Certainly a few	3 c.c. water + 1 c.c. broth, heated for 5 min. at 50° C.
	80	2 R	4	1	39	3	1 c.c. ,, ,, ,, ,,
	81	2 R	4	3	10	4	3 c.c. ,, ,, 2 min. at 70° C.
	82	2 R	4	1	30	10	1 c.c. ,, ,, ,, ,,
	83	2 R	4	3	7	3 or 4	3 c.c. ,, ,, 2 min. at 90° C.
	84	2 R	4	1	10	6	1 c.c. ,, ,, ,, ,,

* For explanation of the system of naming the flasks containing the experimental waters, see Note, p. 185.

Table II—*continued.*

Dates on which plates were poured.	Number of plate.	Particular flask employed.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies from 1 c.c. of water. Total number.	Number of colonies from 1 c.c. of water. Number of anthrax.	Remarks.
				c.c.			
6.4.92	86	2 I	6	3	56	2	3 c.c. water + 1 c.c. broth, heated for 2 min. at 70° C.
	87	2 I	6	1	30	6 or 7	1 c.c. ,, ,, ,, ,,
	88	2 I	6	3	19	0	3 c.c. ,, ,, 2 min. at 90° C.
	89	2 I	6	1	23	2	1 c.c. ,, ,, ,, ,,
3.5.92	167	2 I	3	1	Too much liquefied	0	
	168	2 I	3	$\frac{3}{13}$	21,210	0	
	171	2 I	4	$\frac{1}{75}$	9,500	0	
	172	2 I	4	$\frac{1}{250}$	9,500	0	
10.5.92	180	2 I	5	3	18	8	3 c.c. water + 1 c.c. broth, heated for 2 min. at 70° C.
	181	2 I	5	2	29	9	2 c.c. ,, ,, ,, ,,
24.6.92	290	2 I	3	2	5,778	0	
	291	2 I	3	$\frac{6}{11}$	6,454	0	
	294	2 I	4	$\frac{1}{75}$	6,200	0	
	295	2 I	5	$\frac{1}{375}$	9,000	0	
	298	2 I	4	3	Too much liquefied	0	3 c c. water + 1 c.c. broth, heated for 2 min. at 70° C.
	299	2 I	6	2	16	0	2 c.c. ,, ,, ,, ,,
	302	3 R	3	2	1,650	0	
	303	3 R	3	$\frac{6}{13}$	2,290	0	
	306	3 R	4	$\frac{1}{65}$	2,700	0	
	307	3 R	5	$\frac{1}{325}$	3,250	0	
	310	3 R	5	3	9	0·3	3 c.c. water + 1 c.c. broth, heated for 2 min. at 70° C.
	311	3 R	6	2	15	0	2 c.c. ,, ,, ,, ,,
9.7.92	349	3 I	5	3	10	0·3	3 c.c. water + 1 c.c. broth, heated for 2 min. at 70° C.
	350	3 I	5	2	13	1	2 c.c. ,, ,, ,, ,,
	351	3 R	4	3	11	0	3 c.c. ,, ,, ,, ,,
	352	3 R	5	2	16	1·5	2 c.c. ,, ,, ,, ,,

80,000 were found in the incubated, and upwards of 100,000 in the refrigerated, water. After 46 days (May 3, 1892) they numbered about 13,000 in the incubated water, whilst after 98 days (June 24, 1892) the numbers in the incubated water were about 7,000, and from 2,000 to 3,000 in the refrigerated. These latter figures are much the same as were obtained in the uninfected waters (see Table I) after upwards of 7 months.

3. *Bacteriological Examination of the Unsterilised Thames Water (First Series), Filtered through Swedish Paper and Infected with Anthrax.*

The experiments with the Thames water which had been filtered through Swedish paper prior to infection with anthrax, and which are recorded in Table III (pp. 198 and 199), yielded very much the same results as those made with the unfiltered Thames water recorded in Table II. We find a similar multiplication followed by diminution in the number of water bacteria, both in the flasks kept at summer and winter temperatures respectively. In no single instance was anthrax detected by ordinary plate cultivation, but in the refrigerated water it was found by the special method after 98 days (June 24, 1892), whilst with the incubated water the same method failed to find anthrax after 53 days (May 10, 1892), and again after 98 days (June 24, 1892), whilst in two final examinations, after 113 days (July 9, 1892), one again gave a negative result, whilst the other yielded a feeble growth, presenting a very doubtful resemblance to anthrax. It may be taken, therefore, that in the water preserved at summer temperature the degeneration of anthrax was markedly more rapid than in that kept at the winter temperature.

This infected paper-filtered Thames water was also examined for virulence by direct experiment, as follows:—

Animal Experiment No. 8.—On October 15, 1892, 1 c.c. of water from the flask "1 R, paper-filtered Thames water, infected with anthrax, March 18, 1892," was subcutaneously injected into a white mouse. The mouse died within 4 days 17 hours; there was very extensive œdema, and the spleen was not much enlarged; no bacilli were microscopically found in the spleen, but the characteristic growth was obtained on cultivation in gelatine, thus leaving no doubt that the animal had succumbed to anthrax, although in an attenuated form.

The result of this experiment is interesting in several respects. Thus, firstly, it shows that in this water *anthrax was still present, after 7 months, and in sufficient numbers in 1 c.c. to cause the death of the mouse, whilst it will be remembered that in the unfiltered Thames water this was not the case, so that apparently the removal of a certain proportion of the water bacteria by paper filtration had been conducive to the preservation of the anthrax.*

Table III.—Anthrax in Thames Water. First Series of Experiments. Paper Filtered Thames Water, Infected with Anthrax, March 18, 1892.

Date when plates were poured.	Particular flask employed. *	Number of plate.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies from 1 c.c. of water.		
					Total number.	Number of anthrax colonies.	
				c.c.			
22.3.92	1 I	38	3	$\frac{5}{12}$	Too much liquefied	0	
	1 I	39	3	$\frac{1}{12}$	,,	0	
	1 R	40	3	$\frac{3}{16}$	,,	0	
	1 R	41	3	$\frac{1}{16}$	,,	0	
	1 I	46	3	$\frac{1}{55}$	,,	0	
	1 I	47	3	$\frac{3}{550}$	385,000	0	
	1 R	48	3	$\frac{1}{65}$	Too much liquefied	0	
	1 R	49	3	$\frac{3}{650}$	677,000	0	
3.5.92	2 I	169	3	1	Too much liquefied	0	
	2 I	170	3	$\frac{3}{10}$	3,330	0	
	2 I	170	4	$\frac{1}{75}$	2,250	0	
	2 I	173	4	$\frac{1}{250}$	3,750	0	
10.5.92	2 I	182	5	3	2	0	3 c.c. water + 1 c.c. broth, heated for 2 min. at 70° C.
	2 I	183	5	2	4	0	2 c.c. ,, ,, ,,
24.5.92	1 R	215	3	$\frac{10}{13}$	Too much liquefied	0	
	1 R	216	3	$\frac{3}{13}$	,,	0	
	1 R	219	6	$\frac{1}{60}$	,,	0	
	1 R	220	6	$\frac{1}{250}$	,,	0	

24.6.92	2 I	292	3	2	Too much liquefied	0	The plates were so much liquefied that the estimated numbers are uncertain.
	2 I	293	3	$\frac{1}{2}$	11,160	0	
	2 1	296	4	$\frac{1}{75}$	16,400	0	
	2 1	297	4	$\frac{1}{375}$	15,400	0	
24.6.92	2 1	300	4	3	Too much liquefied	0	3 c.c. water + 1 c.c. broth, heated for 2 min. at 70° C.
	2 I	301	4	2	11	0	2 c.c. ,, ,, ,, ,,
	1 R	304	3	2	Too much liquefied	0	
	1 R	305	3	$\frac{1}{2}$	3,212	0	
	1 R	308	4	$\frac{1}{55}$	5,400	0	
	1 R	309	4	$\frac{1}{275}$	4,700	0	
	1 R	312	7	3	5	3	3 c.c. ,, ,, ,, ,,
	1 R	313	7	2	6	3	2 c.c. ,, ,, ,, ,,
9.7.92	2 I	353	4	3	2	0	3 c.c. water + 1 c.c. broth, heated for 2 min. at 70° C.
	2 I	354	10	2	17	1	2 c.c. ,, ,, ,, ,,

* For explanation of the system of naming the flasks containing the experimental waters, see Note, p. 185.

That the anthrax had undergone considerable attenuation through its long residence in this unsterile water appears from its tardy causation of death with the non-typical symptom of but slight enlargement of the spleen.

4. *Bacteriological Examination of the Infected Thames Water Sterilised by Filtration through Porous Porcelain and by Steam respectively (First Series).*

The results recorded in Tables IV and V may be conveniently considered together. These tables exhibit the effect of introducing a small number of spore-bearing anthrax bacilli into Thames water previously sterilised, on the one hand by filtration through porous porcelain, and on the other by steam.

In these waters the recognition and numeration of the anthrax colonies in the plate cultivations is, of course, attended with no difficulty, and, indeed, it is only by means of these that an estimate of the number of anthrax organisms introduced into the unsterilised water can be formed. It will be seen that the number of anthrax germs introduced into each cubic centimetre of the waters of this Series I amounted to from 30 to 100. The fate of these in the unsterilised water we have already traced; in these sterilised waters it will be seen that they undergo little or no change in numbers during a period of upwards of 3 months, nor is there any material difference in their deportment in the waters sterilised in the two different ways.

Although these waters, which had been submitted to steam and filtration through porcelain respectively, were sterile in the first instance, it was only to be expected that in repeatedly opening the flasks for the purpose of preparing the plate cultivations, some would become contaminated with air-carried bacteria, &c., the presence of such intruding forms in a few of the flasks will be found recorded in the tables, but they generally gave rise to no difficulty in connexion with the plate cultivations.

It will be seen that in the case of these sterilised waters there is evidence of a very slight increase in the number of anthrax colonies after the first day, the number subsequently falling to about the original.

At the foot of Table IV will be seen the result of applying the special method of anthrax identification, which had to be relied on exclusively in the case of the unsterilised waters. On comparing the number of anthrax colonies found by this method with that obtained by ordinary plate cultivation, it will be seen that the greater portion of the anthrax organisms are either actually destroyed in the process of heating to 70° C., for 2 minutes, or, at any rate, are so far enfeebled that they do not subsequently develop in the gelatine. Thus, whilst

Table IV.—Anthrax in Thames Water. First Series of Experiments. Porcelain Filtered Thames Water, Infected with Anthrax, March, 18, 1892.

Date on which plates were poured.	Particular flask employed. *	Number of plate.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies from 1 c.c. of water.		
					Total.	Anthrax.	
				c.c.			
18.3.92	..	3	8	$\frac{2}{3}$	72	72	
	..	4	8	$\frac{1}{3}$	63	57	
22.3.92	1 I	28	6	$\frac{3}{4}$	128	128	
	1 I	29	6	$\frac{5}{12}$	131	131	
	1 R	32	6	$\frac{2}{3}$	102	102	
	1 R	33	6	$\frac{5}{12}$	86	86	
3.5.92	1 I	177	7	2	144	144	
	1 I	178	7	$\frac{3}{11}$	125	125	
24.5.92	1 R	211	6	$1\frac{1}{11}$	Too much liquefied through 1 liquid	..	The water in flask had become contaminated by foreign organisms.
	1 R	212	6	$\frac{3}{11}$	30,287	55	
16.6.92	1 I	259	5		Contaminated with a mould	..	Flask contaminated with a mould.
	1 I	260	5	1	,,	..	
	1 R	263	5	3	Contaminated with a micrococcus		
	1 R	264	5	1	,,		

* For explanation of the system of naming the flasks containing the experimental waters, see Note, p. 185.

Table IV—*continued*.

Date on which plates were poured.	Particular flask employed. *	Number of plate.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies from 1 c.c. of water.		
					Total.	Anthrax.	
29.6.92	2 I	326	6	3	Too much liquefied	..	Contaminated.
	2 I	327	6	1	,,	..	,,
	1 R	328	6	3	..	43	,,
	1 R	329	6	1	..	44	,,
9.7.92	2 I	356	7	3	133	1	3 c.c. water + 1 c.c. broth, heated for 2 min. at 70°C.
	2 I	357	4	2	11	0	2 c.c. ,, ,, ,, ,,
	1 R	358	10	3	11	2	3 c.c. ,, ,, ,, ,,
	1 R	359	10	2	15	5	2 c.c. ,, ,, ,, ,,

* For explanation of the system of naming the flasks containing the experimental waters, see Note, p. 185.

Table V.—Anthrax in Thames Water. First Series of Experiments. Steam-sterilised Thames Water, Infected with Anthrax, March 18, 1892.

Date on which plates were poured.	Particular flask employed. *	Number of plate.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies from 1 c.c. of water. Total.	Anthrax.	
				c.c.			
18.3.92	..	1	8	$\frac{2}{3}$	32	30	
	..	2	8	$\frac{1}{3}$	42	39	
22.3.92	1 I	26	6	$\frac{4}{7}$	Innumerable	112	Contaminated with a micrococcus.
	1 I	27	6	$\frac{3}{7}$	,,	Small No.	
	1 R	30	6	$1\frac{3}{11}$	60	60	
	1 R	31	6	$\frac{5}{11}$	70	70	
3.5.92	1 I	175	7	2	Too much liquefied through contamination		
	1 I	176	7	$\frac{1}{4}$	,,		
24.5.92	1 R	209	8	$\frac{4}{5}$	36	36	
	1 R	210	8	$\frac{1}{5}$	25	20	
16.6.92	2 I	257	6	3	33	33	
	2 I	258	6	1	41	40	
	1 R	261	6	3	45	45	
	1 R	262	6	1	46	46	

* For explanation of the system of naming the flasks containing the experimental waters, see Note, p. 185.

43—44 colonies per 1 c.c. were found in one flask (1 R, porcelain, Table IV) by ordinary plate cultivation, only 2—5 colonies in the same volume were found after the preliminary heating to 70° C. for 2 minutes. On comparing these results with those obtained by the same method in the case of the unsterilised waters, it will be seen that there is distinct evidence of the anthrax organs undergoing more rapid degeneration in the latter than in the sterilised waters. The degeneration is, however, in any case, only an extremely slow one.

There is hardly any difference to be found between the results yielded by the sterile waters kept at summer and winter temperatures respectively, but such slight difference as there is points rather to the degeneration of the anthrax being more retarded at the low than at the higher temperature.

One of these waters was also examined for virulence in October, or 7 months after infection with anthrax, and the following results obtained :—

Animal Experiment No. 9.—On October 15, 1892, 1 c.c. of water from the flask "1 R, Thames water, porcelain-filtered, infected with anthrax, March 18, 1892," was subcutaneously injected into a white mouse. The mouse died within 4 days 17 hours. Anthrax bacilli were found in the spleen and by cultivation in gelatine. There was extensive œdema, and the spleen was not much enlarged.

Thus, in the water sterilised by filtration through porous porcelain the anthrax was still present after 7 *months, in sufficient numbers for* 1 *c.c. to cause the death of the mouse, although from the comparatively slow action and non-typical symptoms it had apparently become somewhat attenuated.*

I did not consider it necessary to make a corresponding experiment with the steam-sterilised Thames water, as, owing to the similarity between the results obtained by plate cultivation from both types of sterilised water, it appeared only reasonable to assume that their virulent effect would also be much the same.

5. *Vitality and Virulence of Anthrax in Thames Water (First Series) Exposed to Diffused Daylight.*

In all the experiments hitherto referred to, the waters were preserved in total darkness, the flasks containing them being placed in an incubator and refrigerator respectively. Further experiments were, however, made with the same waters, exposed to diffused daylight, at the ordinary temperature of the laboratory, and others, again, in which the flasks were exposed to direct sunshine.

The results of the experiments with Thames water (First Series), exposed to diffused daylight, are recorded in Table VI.

All the flasks employed in these experiments had been in the

refrigerator or incubator from the day of infection with anthrax (March 18, 1892) until March 25, 1892, from when they remained in a dark room until April 9, 1892, after which they were exposed to the diffused daylight in a room with a southern aspect.

An inspection of Table VI at once shows that the anthrax in the previously sterilised (porcelain and steam) Thames water survives this exposure to diffused daylight, nor does the number of colonies obtained on plate cultivation differ materially from that obtained from the corresponding flasks maintained throughout in the dark.

On the other hand, the degeneration of the anthrax in the unsterilised Thames water is distinctly more rapid in these flasks exposed to daylight than in those preserved in the dark. Thus, in the case of the unfiltered Thames water (daylight) the special method of examination revealed no anthrax from May 17, 1892, whilst in the same water, kept both in the incubator and refrigerator, anthrax was discovered by the same method on July 9, 1892.

The following experiments were made to test the virulence of the flasks which had been thus exposed to diffused daylight:—

Animal Experiment No. 5.—On October 8, 1892, 1 c.c. of water from the flask "1 I, Thames water, unfiltered, infected with anthrax on March 18, 1892, and exposed to daylight since April 9, 1892," was subcutaneously injected into a white mouse. The mouse did not succumb, but is alive to the present time (November 11, 1892), or 32 days after the operation.

This result was to be anticipated, seeing that the corresponding flasks 3 I and 3 R, which had not been exposed to daylight, also failed to kill mice (see Animal Experiments Nos. 1 and 2).

Animal Experiment No. 3.—On October 7, 1892, 1 c.c. of water from the flask "5 I, Thames water, paper-filtered, which had been infected with anthrax on March 18, 1892, and exposed to daylight since April 9, 1892," was subcutaneously injected into a white mouse. The mouse did not die, but is still alive, 33 days after the operation.

It will be remembered that a corresponding flask, 1 R, which had not been exposed to daylight did kill a mouse (see Animal Experiment No. 8), so that the virulence has in this case been reduced by the exposure.

Animal Experiment No. 4.—On October 7, 1892, 1 c.c. of water from the flask "5 I, Thames water, porcelain-filtered, which had been infected with anthrax on March 18, 1892, and exposed to daylight since April 9, 1892," was subcutaneously injected into a white mouse. The mouse died within 6 days $20\frac{1}{2}$ hours. The body exhibited extensive œdema; the spleen was only slightly enlarged, but was found to contain anthrax bacilli both microscopically and by cultivation in gelatine.

Animal Experiment No. 10.—On October 15, 1892, 1 c.c. of water

from the flask " 5 I, Thames water, steam-sterilised, which had been infected with anthrax on March 18, 1892, and exposed to daylight since April 9, 1892," was subcutaneously injected into a white mouse. The mouse died within 4 days 17 hours; the body exhibited much œdema and the spleen was not very large; anthrax bacilli were detected in the latter both with the microscope and by cultivation in gelatine.

The contrast exhibited by the sterilised and unsterilised Thames water is thus again most striking in the case of these flasks exposed to daylight, for both the unfiltered and paper-filtered waters failed to kill, whilst the porcelain, filtered and the steam-sterilised waters were fatal to the mice into which they were injected. The lethal effect of both the latter, and especially of the porcelain-filtered water, accompanied by the non-typical symptom of only slight enlargement of the spleen, points again to an attenuation of the virus.

These results did not lead me to conclude, however, that the anthrax virus was necessarily quite extinct in these two unsterilised waters (viz., the unfiltered and paper-filtered Thames water), and I resorted, therefore, to the method before employed (see p. 194) of revivifying it by the addition of 5 c.c. of sterile broth to each of the two flasks in question. The flasks so treated were placed in an incubator at 37° C., and the following further experiments made with them:—

Animal Experiment No. 20.—On October 22, 1892, 0·5 c.c. of the water (to which broth had been added on October 15, 1892) in the flask " 1 I, Thames water, unfiltered, and infected with anthrax on March 18, 1892, exposed to daylight since April 9, 1892," was subcutaneously injected into a white mouse. The mouse died within 2 days 18 hours. The body exhibited extensive œdema and the spleen was much enlarged; the latter was found full of anthrax bacilli, the presence of which was confirmed by cultivation in gelatine.

Animal Experiment No. 17.—On October 18, 1892, 0·5 c.c. of the water (to which broth had been added on October 15, 1892) in the flask " 5 I, Thames water, paper-filtered, and infected with anthrax March 18, 1892, exposed to daylight since April 9, 1892," was subcutaneously injected into a white mouse. The mouse died within 1 day 19 hours. Only few bacilli were found in the spleen, but more in the kidney; their presence was confirmed by gelatine cultivations from both organs.

These experiments show, then, *that in the flasks in question (unsterilised Thames water exposed to daylight), although the number of anthrax germs had been so far reduced that* 1 *c.c. would not kill mice, yet after nourishment with broth they were so revivified as to be fatal to these animals when injected in the same or even a smaller quantity.*

Table VI.—Anthrax in Thames Water. First Series of Experiments. Diffused Daylight Experiments, Infected March 18, 1892.

Date on which plates were poured.	Number of plate.	Particular flask and water employed. *	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies from 1 c.c. of water. Total.	Number of anthrax.	The flasks were exposed to diffused daylight from 9.4.92 onwards.
		Unfiltered.		c.c.			
12.5.92	184	1 I	4	$\frac{5}{6}$	Too much liquefied	0	
	185	1 I	4	$\frac{1}{4}$	,,	0	
	188	1 I	4	$\frac{1}{60}$	,,	0	
	189	1 I	4	$\frac{1}{200}$	,,	0	
17.5.92	180*a*	1 I	4	2	8	0	2 c.c. water + 1 c.c. broth, heated for 2 min. at 70° C.
24.6.92	314	1 I	3	2	Too much liquefied	0	
	315	1 I	3	$\frac{1}{2}$	,,	0	
	318	1 I	4	$\frac{1}{55}$	15,300	0	
	319	1 I	4	$\frac{1}{275}$	17,350	0	
	322	1 I	6	3	8	0	3 c.c. water + 1 c.c. broth, heated for 2 min. at 70° C.
	323	1 I	4	2	8	0	2 c.c. ,, ,, ,, ,,
		Paper filtered.					
12.5.92	186	5 I	4	$\frac{5}{6}$	Too much liquefied	0	
	187	5 I	4	$\frac{1}{4}$	,,	0	
	190	5 I	4	$\frac{1}{60}$	,,	0	
	191	5 I	4	$\frac{1}{200}$	,,	0	
17.5.92	182	5 I	4	2	5	1	2 c.c. water + 1 c.c. broth, heated for 2 min. at 70° C.

* For explanation of the system of naming the flasks containing the experimental waters, see Note, p. 185.

Table VI—*continued.*

Date on which plates were poured.	Number of plates.	Particular flask and water employed. *	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies from 1 c.c. of water.		The flasks were exposed to diffused daylight from 9.4.92 onwards.
					Total.	Number of anthrax.	
		Paper filtered.		c.c.			
24.6.92	316	5 I	3	$2\frac{1}{2}$	Too much liquefied	0	
	317	5 I	3	$\frac{1}{2}$	”	0	
	320	5 I	3	$\frac{1}{48}$	15,600	0	
	321	5 I	4	$\frac{1}{240}$	12,000	0	
	324	5 I	4	$3\frac{1}{3}$	4	0	$3\frac{1}{3}$ c.c. water + 1 c.c. broth, heated for 2 min. at 70° C.
	325	5 I	7	$2\frac{1}{6}$	2	1	$2\frac{1}{6}$ c.c. ” ” ” ”
		Porcelain filtered.					
12.5.92	194	5 I	15	$1\frac{4}{11}$	22	22	Plate partially liquefied at edges, hence result only approximate.
	195	5 I	15	$\frac{3}{11}$	66	66	
16.6.92	255	5 I	8	3	..	20	Contaminated with a micrococcus.
	256	5 I	8	1	..	43	” ” ”
		Steamed.					
12.5.92	192	5 I	15	$1\frac{3}{11}$	27	27	
	193	5 I	15	$\frac{3}{11}$	22	22	
16.6.92	253	5 I	8	3	3,502	34	Contaminated with a bacillus.
	254	5 I	8	1	4,554	35	” ” ”

* For explanation of the system of naming the flasks containing the experimental waters, see Note, p. 185.

6. *Vitality and Virulence of Anthrax in Thames Water (First Series) Exposed to Direct Sunshine.*

The effect of direct sunshine is recorded in Table VII. The flasks were taken from the incubator and refrigerator respectively on March 25, 1892; they remained in a dark room from that day to April 9, 1892, and from then onwards they were exposed to as much sunshine as could be conveniently obtained, and which was approximately estimated in hours, although it is obviously very difficult to make any exact determination of the latter. The conditions of experiment are, of course, also much complicated by the fact that the temperature of the water so exposed was subject to very great variation, although it certainly never exceeded 32° C.

The results, which are very striking, are easily followed by reference to Table VII.

From the table it will be seen that in—

Unfiltered Thames water, anthrax was still alive on May 2, 1892, after 56 hours' sunshine, but extinct on May 12, 1892, after about 84 hours' insolation.

Paper-filtered Thames water, anthrax was almost extinct on May 15, 1892, after about 92 hours' insolation, and quite extinct on June 17, 1892, after about 151 hours' sunshine.

Thames water filtered through porcelain, anthrax was still alive on May 2, 1892, after about 56 hours of sun, but extinct on May 12, 1892, after about 84 hours' insolation.

Thames water sterilised with steam, anthrax was still alive on May 2, 1892, after about 56 hours', but dead on May 12, 1892, after about 84 hours' sunshine.

In consequence of the sunshine having destroyed the greater number of those water bacteria causing liquefaction of the gelatine, it was possible to incubate the plates for a long period of time, and thus in most instances to dispense with the special method of examination by preliminary heating already so often referred to.

The above results have only reference to the presence or absence of anthrax as revealed by cultivation, but experiments were also made on the virulence of these waters which had been exposed to direct insolation. Thus:—

Animal Experiment No. 30.—On November 2, 1892, 1 c.c. of the water from the flask "4 I, Thames water, unfiltered, and infected with anthrax on March 18, 1892, exposed to 151 hours' sunshine," was subcutaneously injected into a white mouse. The mouse is still alive (November 14, 1892).

Animal Experiment No. 31.—On November 2, 1892, 1 c.c. of the water from flask "4 I, Thames water steam-sterilised, and infected with anthrax on March 18, 1892, exposed to 151 hours' sunshine,"

Table VII.—Anthrax in Thames Water. First Series of Experiments. Sunlight Experiments, Thames Water infected with Anthrax, March 18, 1892.

Water.	Date when plates were poured.	Particular flask used. *	Number of plate.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies in 1 c.c. of the water. Total.	Anthrax.	The flasks were exposed from 9.4.92 onwards.	
					c.c.				
Unfiltered	2.5.92	4 I	151	5	1	Too much liquefied	0	. ..	56 hours' sunshine.
		4 I	152	5	$\frac{3}{13}$	700	0	.. ..	
		4 I	155	8	$\frac{1}{55}$	Too much liquefied	0	.. ..	
		4 I	156	8	$\frac{3}{550}$	10,000	550	Much importance cannot be attributed to the exact number in this case, as only 3 anthrax colonies were found on the plate, so that there is much inaccuracy possible in multiplying up to 1 c.c.	
	12.5.92	4 I	196	15	$1\frac{2}{13}$	Too much liquefied	0	84 hours' sunshine.	
		4 I	197	15	$\frac{3}{13}$	,,	0		
		4 I	200	13	$\frac{6}{325}$	33,300	0		
		4 I	201	13	$\frac{3}{650}$	9,750	0		
	17.6.92	4 I	277	8	1	Not counted owing to gelatine liquefying	0	.. ..	151 hours' sunshine.
		4 I	278	8	$\frac{3}{13}$	,,	0	.. ..	
		4 I	281	12	$\frac{1}{75}$	Too much liquefied	0	.. ..	
		4 I	282	8	$\frac{1}{375}$	33,550	0	.. ..	
		4 I	286	12	3	500	0	3 c.c. water + 1 c.c. broth, heated for 2 min. at 70° C.	
		4 I	287	14	1	33	0	1 c.c. ,, ,, ,,	
Paper filtered	2.5.92	4 I	153	5	1	3,970	0	These were counted prematurely for fear of liquefaction.	56 hours' sunshine.
		4 I	154	5	$\frac{1}{5}$	270	0		
		4 I	157	8	$\frac{1}{55}$	9,500	100	Only 1 and 2 anthrax colonies respectively were found on the plate, so that there is much chance of error in multiplying up to 1 c.c.	
		4 I	158	8	$\frac{3}{550}$	2,200	200		

	15.5.92	4 I	198	15	1	7,630	2	92 hours' sunshine.
		4 I	199	15	$\frac{1}{5}$	4,500	5	
		4 I	202	13	$\frac{2}{125}$	19,150	0	
	17.6.92	4 I	279	8	1	11,900	0	.. .. (151 hours' sunshine.)
		4 I	280	8	$\frac{3}{11}$	18,260	0	.. ..
		4 I	283	12	$\frac{1}{55}$	Too much liquefied	0	.. ..
		4 I	284	12	$\frac{1}{275}$	41,500	0	.. ..
		4 I	288	14	3	1	0	3 c.c. water + 1 c.c. broth, heated for 2 min. at 70° C.
		4 I	289	14	1	4	0	1 c.c. ,, ,, ,,
Porcelain filtered	2.5.92	4 I	161	7	2	Too much liquefied	..	56 hours' sunshine.
		4 I	162	7	$\frac{3}{11}$	18	7	
	12.5.92	4 I	206	13	$1\frac{1}{3}$	2	0	84 hours' sunshine.
		4 I	207	13	$\frac{1}{3}$	0	0	
	16.6.92	4 I	251	15	3	16	0	151 hours' sunshine.
		4 I	252	15	1	10	0	
Steamed .	2.5.92	4 I	159	7	2	Too much liquefied	..	56 hours' sunshine.
		4 I	160	7	$\frac{1}{4}$	16	4	
	12.5.92	4 I	204	13	$1\frac{1}{2}$	2	0	84 hours' sunshine.
		4 I	205	13	$\frac{3}{10}$	0	0	
	16.6.92	4 I	249	15	3	2	0	151 hours' sunshine.
		4 I	250	15	1	1	0	

Note.—Flasks up to 2.5.92 had received 56 hours' sunshine.
,, ,, 12.5.92 ,, ,, 84 ,, ,,
,, ,, 15.5.92 ,, ,, 92 ,, ,,
,, ,, 16.6.62 ,, ,, 151 ,, ,,
,, ,, 17.6.92 ,, ,, 151 ,, ,,
Temperature—Lowest 8° C.
Highest 32 ,,

* For explanation of the system of naming the flasks containing the experimental waters, see Note, p. 185.

was subcutaneously injected into a white mouse. The mouse is still alive (November 14, 1892).

Animal Experiment No. 32.—On November 2, 1892, 1 c.c. of the water from flask "4 I, Thames water, porcelain-filtered, and infected with anthrax on March 18, 1892, exposed to 151 hours' sunshine," was subcutaneously injected into a white mouse. The mouse is still alive (November 14, 1892).

Thus, in all three cases, the water was non-virulent when injected to the amount of 1 c.c.; this is, it will be observed, the first instance in which the sterilised waters infected with anthrax had become non-virulent. It was, however, obviously not to be necessarily concluded that the anthrax had become absolutely extinct in these waters, and in order to put this point to the test the flasks in question were each treated with 5 c.c. of sterile broth and incubated at 37° C., after which the following further experiments were made :—

Animal Experiment No. 36.—On November 9, 1892, 0·5 c.c. of the water (to which broth was added on November 7, 1892) from flask "4 I, Thames water, unfiltered, and infected with anthrax on March 18, 1892, exposed to 151 hours' sunshine," was subcutaneously injected into a white mouse. The mouse is still alive (November 14, 1892).

Animal Experiment No. 37.—On November 9, 1892, 0·6 c.c. of the water (to which broth was added on November 7, 1892) from flask "4 I, Thames water, steam-sterilised, and infected with anthrax on March 18, 1892, exposed to 151 hours' sunshine," was subcutaneously injected into a white mouse. The mouse is still alive (November 14, 1892).

Animal Experiment No. 38.—On November 9, 1892, 0·6 c.c. of the water (to which broth was added on November 7, 1892) from flask "4 I, Thames water, poreelain-filtered, and infected with anthrax on March 18, 1892, exposed to 151 hours' sunshine," was subcutaneously injected into a white mouse. The mouse is still alive November 14, 1892).

N.B.—These three mice, Nos. 36, 37, and 38, all lived much longer than November 14, 1802, so there can be no doubt that they escaped infection.

Thus, in these waters exposed to direct sunshine, the anthrax germs were completely destroyed and could not be revived by the addition of broth.

The destruction of anthrax spores by direct sunshine is a subject which has received the attention of a number of observers. Thus, Arloing ('Compt. Rend.,' vol. 100, 1885, p. 378, and vol. 101, p. 511) found that they were destroyed in two hours, whilst in subsequent experiments in which the spores were placed in broth maintained at a temperature of 4—11° C. by means of ice five hours' insolation

effected their destruction. Roux ('Ann. de l'Inst. Past.,' 1887, p. 445) again insolated the spores when dispersed in the aqueous humour of the ox-eye, and found them destroyed in from twenty-nine to fifty-four hours, whilst Pansini ('Rivista d'Igiene,' 1889) observed their destruction on potatoes in from four to five hours, in gelatine in from six to seven hours, and in broth in from thirty minutes to two hours. In all these experiments it will be seen that nutrient culture media were employed for the insolation, and that the spores were destroyed in a much briefer period of time than in my experiments, in which they were insolated in Thames water. This same phenomenon of *the spores of anthrax being more resistant to the action of sunshine in water than in ordinary culture materials* has also been observed by Straus ('Société de Biologie,' 1886, p. 473) and by Momont ('Ann. de l'Inst. Past.,' 1892, p. 21), who both, however, appear to have made use of distilled water only.

II. Vitality and Virulence of Anthrax Spores in Thames Water (Second Series of Experiments).

Owing to the very small number of anthrax germs introduced into the water in the First Series of experiments, it was deemed advisable to carry out a Second Series in which a much larger number were inoculated into the several waters, whilst as a further modification and check, the infection was made with virulent anthrax from a totally different source to that employed in the First Series.

The infection was made as follows:—

50 c.c. of Thames water previously steam sterilised were placed in a small sterile stoppered bottle, and inoculated with 5 needle loops from an anthrax culture in glycerine agar of 3 weeks' age, and with 4 needle loops of another similar culture of $3\frac{1}{2}$ weeks' age. The water thus infected was then violently shaken, after which three portions of 5 c.c. each were measured with a sterile pipette into three flasks containing the three following waters respectively:—

(*a.*) Unfiltered Thames water (750 c.c.).
(*b.*) Thames water sterilised by filtration through porous porcelain (1000 c.c.).
(*c.*) Thames water sterilised by steam (1000 c.c.).

The waters thus infected were violently agitated to secure even distribution of the anthrax, after which each water was divided amongst a number of small flasks as follows:—

(*a.*) Infected unfiltered water	3 small flasks for incubator (18—20° C.).	3 ,, ,, refrigerator (6—10° C.).
(*b.*) Infected porcelain-filtered water ..	3 small flasks for incubator.	3 ,, ,, refrigerator.
(*c.*) Infected steamed water	3 small flasks for incubator.	3 ,, ,, refrigerator.

The contents of these flasks were then from time to time submitted to bacteriological examination in the same way as in the First Series of experiments.

1. *Infected Unfiltered Thames Water* (*Second Series*).

The results of the experiments made with the unfiltered Thames water are recorded in Table VIII.

The plates poured on the day of infection (March 25, 1892) yielded about 15,000 colonies per cubic centimetre, of which about 12,000 were easily identifiable as anthrax.

The second series of plates were poured 4 days later (March 29, 1892), but those both from the incubator and the refrigerator flask were already so badly liquefied on the third day of incubation that it was impossible either to count or identify any anthrax colonies.

In consequence of this failure of the ordinary plate cultivation method, the special process for anthrax identification by preliminary heating was resorted to on April 13, 1892, for one of the refrigerator flasks, with the result that anthrax was easily recognised.

Ordinary plates were again poured some days later, on April 26, 1892, both from an incubator and a refrigerator flask, but in neither case could any anthrax colonies be found.

Ordinary plates were again poured on June 17, 1892, but only in one out of eight plates could any anthrax colonies be identified; nevertheless, when on the same day the special method by preliminary heating to 70° C. was adopted, a number of anthrax colonies were easily obtained both from the incubator as well as the refrigerator flask. This conclusively demonstrates the necessity of resorting to the special method of examination, and of not relying on that by ordinary plate cultivation.

The anthrax had thus retained its vitality in the unfiltered Thames water for 84 days, both when the water was maintained at a summer and a winter temperature respectively, although it was undoubtedly present in very much diminished numbers compared with those originally introduced.

Some of the flasks were finally examined on October 8, or 197 days after the original infection, and anthrax was still discoverable by the special method, although in slightly smaller numbers than on the previous occasion. By comparing these numbers with those obtained

in a corresponding experiment made on the same day (October 8) with the porcelain-filtered water (which was also orginally infected with about the same number of anthrax germs as the unfiltered water), it will be seen that the numbers have not diminished to nearly the same extent in the porcelain-filtered water as in the unsterilised water; in fact, in the porcelain-filtered water they have undergone hardly any reduction at all.

The following experiments were conducted in order to investigate the virulence of this unfiltered water in which plate cultivation had revealed the presence of living anthrax germs:—

Animal Experiment No. 21.—On October 23, 1892, 1 c.c. of the water in flask "1 I, unfiltered Thames water (Second Series), infected with anthrax, March 25, 1892," was subcutaneously injected into a white mouse. The mouse died within 4 days 16 hours, anthrax bacilli being duly found in the spleen.

Animal Experiment No. 26.—On October 29, 1892, 1 c.c. of the water in flask "2 I, unfiltered Thames water (Second Series), infected with anthrax, March 25, 1892," was subcutaneously injected into a white mouse. The mouse is still alive (November 14, 1892) 16 days after the operation, and therefore out of danger of anthrax.

Animal Experiment No. 25.—On October 29, 1892, 1 c.c. of water from the flask "3 R, unfiltered Thames water (Second Series), infected with anthrax, March 25, 1892," was subcutaneously injected into a white mouse. The mouse died within 5 days 16 hours; the body exhibited extensive œdema, and the spleen was found full of anthrax bacilli, which was further confirmed by the gelatine cultivations prepared from that organ.

Animal Experiment No. 33.—This was performed on November 5, 1892, and was a repetition of Experiment No. 26. The mouse died within 2 days 4 hours. The spleen was much enlarged and found to contain anthrax bacilli, the characteristic growth being obtained in gelatine cultivations from the same organ.

Animal Experiment No. 34.—On November 5, 1892, 1 c.c. of water from the flask "2 R, unfiltered Thames water (Second Series), infected with anthrax, March 25, 1892," was subcutaneously injected into a white mouse. The mouse died within 6 days 16 hours; the body exhibited much œdema; the spleen was considerably enlarged, and an abundance of anthrax bacilli were found in it.

We may conveniently also refer at this point to experiments made with some flasks resembling the above in all respects excepting that from July 23, 1892, onwards, when they were taken out of the incubator and refrigerator respectively, they had been exposed to diffused daylight, whilst the above flasks had been kept in the dark throughout. The results of the plate cultivations from these daylight flasks, which do not call for any special comment beyond that

Table VIII.—Anthrax in Thames Water. Second Series of Experiments. Unfiltered Thames Water, infected with Anthrax, March 25, 1892.

Date on which plates were poured.	Particular flask employed. *	Number of plate.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies from 1 c.c. of water.		Remarks.
					Total.	No. of anthrax.	
				c.c.			
25.3.92	..	58	4	$\frac{1}{4}$	12,320	10,780	This was the same sample of Thames water as was employed in the First Series of experiments.
		59	4	$\frac{1}{12}$	12,324	10,428	
		60	4	$\frac{2}{75}$	19,400	16,700	
		61	4	$\frac{1}{100}$	17,700	12,000	
29.3.92	1 I	70	3	$\frac{2}{15}$	Too much liquefied		
	1 I	71	3	$\frac{1}{15}$	,,		
	1 I	74	3	$\frac{2}{125}$	,,		
	1 I	75	3	$\frac{2}{375}$	,,		
	1 R	72	3	$\frac{1}{6}$	,,		
	1 R	73	3	$\frac{1}{12}$	,,		
	1 R	76	3	$\frac{3}{200}$	,,		
	1 R	77	3	$\frac{1}{200}$	,,		
13.4.92	3 R	91	5	3	72	44	3 c.c. water + 1 c.c. broth heated for 2 min. at 70° C.
	3 R	92	5	1	60	24	1 c.c. ,, ,, ,, ,,
	3 R	93	5	3	12	6	3 c.c. ,, ,, ,, ,,
	3 R	94	7	1	42	8	1 c.c. ,, ,, ,, ,,

26.4.92	1 I	122	3	$\frac{2}{13}$	15,250	0	
	1 I	123	3	$\frac{1}{13}$	14,600	0	
	1 I	126	4	$\frac{1}{60}$	Too much liquefied		
	1 I	127	4	$\frac{1}{300}$	16,500	0	
	1 R	124	4	$\frac{2}{13}$	1,260	0	
	1 R	125	4	$\frac{1}{13}$	1,476	0	
	1 R	128	4	$\frac{1}{60}$	Too much liquefied		
	1 R	129	4	$\frac{1}{300}$	6,900	0	
17.6.92	1 I	265	3	$\frac{1}{6}$	7,344	0	
	1 I	266	3	$\frac{1}{12}$	7,548	0	
	1 I	269	4	$\frac{4}{375}$	9,400	0	
	1 I	270	4	$\frac{1}{375}$	8,250	0	
	1 R	267	3	$\frac{1}{6}$	5,028	0	
	1 R	268	3	$\frac{1}{12}$	4,990	0	
	1 R	271	4	$\frac{1}{75}$	6,250	0	
	1 R	272	4	$\frac{1}{300}$	4,800	1,200	Only 4 anthrax colonies were actually found on the plate, so that, of course, there is much room for inaccuracy in multiplying up to 1 c.c.
17.6.92	1 I	273	7	$3\frac{3}{11}$	32	28	$3\frac{3}{11}$ c.c. water + 1 c.c. broth heated for 2 min. at 70° C.
	1 I	274	7	$1\frac{1}{11}$	62	49	$1\frac{1}{11}$ c.c. water + 1 c.c. broth heated for 2 min. at 70° C.
	1 R	275	4	$2\frac{10}{13}$	12	7	$2\frac{10}{13}$ c.c. water + 1 c.c. broth heated for 2 min. at 70° C.
	1 R	276	4	$\frac{12}{13}$	10	4	$\frac{12}{13}$ c.c. water + 1 c.c. broth heated for 2 min. at 70° C.
8.10.92	2 I	412	5	3	21	3	3 c.c. water + 1 c.c. broth heated for 2 min. at 70° C.
	2 I	413	5	3	28	3	3 c.c. " " " "
	2 R	414	5	3	32	3	3 c.c. " " " "
	2 R	415	5	3	17	13	3 c.c. " " " "

* For explanation of the system of naming the flasks containing the experimental waters, see Note, p. 185.

Table VIII—*continued.*

Date on which plates were poured.	Particular flask employed. *	Number of plate.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies from 1 c.c. of water. Total.	No. of anthrax.	
Diffused Daylight. These flasks were exposed to diffused daylight on July 23, 1892.							
18.10.92	3 I	485	4	3	15	5	3 c.c. water + 1 c.c. broth heated for 2 min. at 70° C.
	3 I	486	4	3	Too much liquefied	. .	3 c.c. ,, ,, ,, ,,
	3 I	489	3	$\frac{1}{6}$	15,480	0	
	3 I	490	3	$\frac{1}{12}$	11,520	0	
	1 R	487	4	3	23	7	3 c.c. ,, ,, ,, ,,
	1 R	488	5	3	81	17	3 c.c. ,, ,, ,, ,,
	1 R	491	3	$\frac{1}{6}$	20,034	0	
	1 R	492	3	$\frac{1}{12}$	20,592		

Temp. of incub. on 22.7.92, 19° C.; on 20.9.92, 17° C.; on 16.10.92, 15° C. Temp. of refrig. on 23.7.92, 9° C.; on 20.10.92, 15° C. The refrigerator was no longer supplied with ice after 23.7.92.

* For explanation of the system of naming the flasks containing the experimental waters, see Note, p. 185.

anthrax was found in both of them, are recorded at the foot of Table VIII. Thus:

Animal Experiment No. 18.—On October 21, 1892, 1 c.c. of the water in flask "3 I, unfiltered Thames water, infected with anthrax, March 25, 1892," was subcutaneously injected into a white mouse. The mouse died within 3 days 17 hours; the spleen was much enlarged, and although no anthrax bacilli could be found either in the latter or in the kidney with the microscope, gelatine cultivations prepared from the spleen developed the characteristic growths in due course, leaving no doubt that the animal had succumbed to anthrax.

Animal Experiment No. 27.—On October 31, 1892, 1 c.c. of water from the flask "1 R, unfiltered Thames water (Second Series), infected with anthrax, March 25, 1892," was subcutaneously injected into a white mouse. The mouse died within 2 days 18½ hours, anthrax bacilli being found in the spleen, and the characteristic growth obtained on gelatine cultivation.

These experiments with the unfiltered Thames water (Second Series) on being contrasted with those of the same water (First Series) show that the virulence was distinctly greater in the Second than in the First Series, for both incubator and refrigerator flasks of the Second Series, irrespectively of whether they had been kept in darkness or in the daylight, were sufficiently virulent to be fatal to mice. That the anthrax must have suffered a certain amount of attenuation is clear from the fact that one of the mice (Experiment No. 26) remained alive after receiving 1 c.c. of the water in flask "2 I," although a second mouse similarly inoculated succumbed. *This unquestionably greater virulence of the unfiltered Thames water (Second Series) is, doubtless, due to the much larger number of anthrax germs with which the water was infected in the Second than in the First Series.* It is particularly interesting that the mouse in Experiment No. 26 remained alive, because from the plate cultivations (see Table VIII) it is perfectly certain that in the 1 c.c. injected a number of living anthrax germs must have been present, and it is obvious, therefore, that their virulence must have been weakened by the long residence in the unsterilised water.

2. *Experiments with Sterilised Thames Water (Second Series).*

The results of the experiments made on Thames water sterilised by filtration through porous porcelain are recorded in Table IX, whilst those on the same water sterilised by steam are given in Table X. From these tables it will be seen that about 6000 anthrax organisms per cubic centimetre were introduced into the porcelain-filtered water, and about 8000 into the steamed water; whilst in Table VIII it was shown that about 12,000 were introduced into the

Table IX.—Anthrax in Thames Water. Second Series of Experiments. Porcelain Filtered Thames Water, infected with Anthrax, March 25, 1892.

Date when plates were poured.	Particular flask employed. *	Number of plate.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies from 1 c.c. of water.		Remarks.
					Total.	Anthrax.	
25.3.92	..	53	4	c.c. $\frac{9}{11}$	Too much liquefied through anthrax colonies		
		54	4	$\frac{4}{11}$	6380	6380	
29.3.92	1 I	64	6	$\frac{6}{13}$	6294	6294	
	1 I	65	6	$\frac{2}{13}$	6058	6045	
	I R	68	6	$\frac{6}{11}$	3600	3600	
	1 R	69	6	$\frac{2}{11}$	3074	3069	
13.4.92	1 R	103	8	$\frac{4}{11}$	2173	2159	
	1 R	104	8	$\frac{8}{11}$	2646	2640	
	1 R	95	5	$\frac{4}{11}$	318	318	$\frac{4}{11}$ c.c. water + 1 c.c. broth, heated for 2 min. at 70° C.
	1 R	96	5	$\frac{8}{11}$	408	402	$\frac{8}{11}$ c.c. " " " "
	1 R	97	5	$\frac{1}{3}$	21	0	$\frac{1}{3}$ c.c. " " " 90° C.
	1 R	98	5	$\frac{2}{3}$	12	12	$\frac{2}{3}$ c.c. " " " "
26.4.92	1 I	132	8	$\frac{1}{2}$	1028	1028	
	1 I	133	8	$\frac{1}{6}$	870	864	
	2 I	140	8	$\frac{1}{2}$	4760	4760	
	2 I	141	8	$\frac{1}{6}$	5592	5592	
	1 R	136	8	$\frac{2}{5}$	2628	2625	
	1 R	137	8	$\frac{2}{15}$	2430	2400	
	2 R	144	8	$\frac{6}{11}$	3172	3170	
	2 R	145	8	$\frac{2}{11}$	2921	2904	

16.6.92	2 I	243	6	$\frac{6}{13}$	6130	6130	Contaminated with a micrococcus.
	2 I	244	6	$\frac{2}{13}$	5909	5909	" " "
	2 R	247	5	$\frac{6}{11}$	3615	3615	
	2 R	248	8	$\frac{2}{11}$	3520	3520	
11.7.92	2 R	349	4	$\frac{6}{13}$	2458	2458	
	2 R	350	8	$\frac{2}{13}$	2756	2587	
	2 R	353	8	3	151	145	3 c.c. water + 1 c.c. broth, heated for 2 min. at 70° C.
	2 R	354	8	2	137	128	2 c.c. " " " "
8.10.92	3 R	416	5	3	92	92	3 c.c. water + 1 c.c. broth, heated for 2 min. at 70° C.
	3 R	417	5	3	104	104	3 c.c. " " " "
	3 R	418	5	$\frac{10}{11}$	3630	3630	
	3 R	419	5	$\frac{3}{11}$	3300	3300	
26.10.92	2 I	528	5	$\frac{3}{8}$	4180	4180	
	2 I	529	5	$\frac{1}{8}$	4784	4784	
	3 R	532	4	$\frac{3}{8}$	3540	3540	
	3 R	533	4	$\frac{1}{8}$	4400	4400	

During the vacation the temperature of the incubator was, 22.7.92, 19° C.; 20.9.92, 70° C.; 16.10.92, 15° C.
" " " " refrigerator was, 23.7.92, 9° C.; 20.10.92, 15° C.
The refrigerator was no longer supplied with ice after 30.7.92.

Diffused Daylight Experiments.

These flasks were placed in diffused daylight on July 23, 1892.

18.10.92	3 I	495	4	$\frac{10}{11}$			Plates were covered with moulds, and, therefore, uncountable.
	3 I	496	4	$\frac{5}{11}$			
	2 R	499	6	$\frac{5}{6}$	1512	1512	
	2 R	500	6	$\frac{5}{12}$	1209	1209	

* For explanation of the system of naming the flasks containing the experimental waters, see Note, p. 185.

Table X.—Anthrax in Thames Water. Second Series of Experiments. Steamed Thames Water, infected with Anthrax, March 25, 1892.

Date on which plates were poured.	Particular flask employed. *	Number of plate.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	No. of colonies from 1 c.c. of water.		Remarks.
					Total.	Anthrax.	
25.3.92		51	4	c.c. $\frac{2}{13}$	Too much liquefied through anthrax		
		52	4	$\frac{4}{13}$	8060	8060	
29.3.92	1 I	62	6	$\frac{6}{11}$	5280	4224	Contaminated with a micrococcus.
	1 I	63	6	$\frac{2}{11}$	8800	6600	” ” ”
	1 R	66	6	$\frac{1}{2}$	4020	4012	
	1 R	67	6	$\frac{1}{6}$	4060	4026	
13.4.92	1 R	105	8	$\frac{1}{3}$	4080	4077	
	1 R	106	8	$\frac{2}{3}$	3822	3822	
	1 R	99	5	$\frac{1}{3}$	1200	1176	$\frac{1}{3}$ c.c. water + 1 c.c. broth heated for 2 min. at 70° C.
	1 R	100	5	$\frac{2}{3}$	1500	1494	$\frac{2}{3}$ c.c. ” ” ” ”
	1 R	101	5	$\frac{1}{3}$	18	18	$\frac{1}{3}$ c.c. ” ” ” 90° C.
	1 R	102	5	$\frac{2}{3}$	75	75	$\frac{2}{3}$ c.c. ” ” ” ” Compare effect of same treatment on porcelain-filtered and unfiltered infected waters.
26.4.92	1 I	130	8	$\frac{3}{8}$	Large number of colonies, including anthrax, but so unevenly distributed as to render accurate numeration impossible	..	Contaminated with a micrococcus.
	1 I	131	8	$\frac{1}{8}$	Ditto		” ”
	2 I	138	8	6	3560	3551	

	2 I	139	8	$\frac{2}{11}$	4318	4312	
	1 R	134	8	$\frac{1}{2}$	3818	3816	
	1 R	135	8	$\frac{1}{6}$	3978	3978	
	2 R	142	8	$\frac{3}{7}$	3397	3390	
	2 R	143	8	$\frac{1}{7}$	3969	3934	
16.6.92	2 I	241	6	$\frac{2}{5}$	6300	6300	
	2 I	242	6	$\frac{2}{15}$	4875	4875	
	2 R	245	8	$\frac{2}{5}$	5250	5250	
	2 R	246	8	$\frac{2}{15}$	5917	5850	
11.7.92	2 R	347	4	$\frac{2}{5}$	3770	3770	
	2 R	348	5	$\frac{2}{15}$	2835	2835	
	2 R	351	8	3	181	179	3 c.c. water + 1 c.c. broth heated for 2 min. at 70° C.
	2 R	352	8	2	168	163	2 c.c. ,, ,, ,, ,,
							These experiments were performed to determine the diminution in the number of anthrax colonies by broth experiments.
26.10.92	1 I	526	5	$\frac{1}{2}$			Contaminated, and impossible to count anthrax colonies.
	1 I	527	5	$\frac{1}{6}$	..	..	,, ,, ,, ,,
	3 R	530	4	$\frac{6}{13}$	3700	3700	
	3 R	531	4	$\frac{2}{13}$	4648	4648	

Diffused Daylight.

Flasks placed in Diffused Daylight, July 23, 1892.

18.10.92	3 I	493	6	$\frac{5}{6}$	3540	3540	
	3 I	494	6	$\frac{5}{12}$	3240	3240	
	2 R	497	5	$\frac{5}{6}$	2736	2736	
	2 R	498	5	$\frac{5}{12}$	2976	2976	

Temperature of incubator, 22.7.92, 19° C.; on 20.9.92, 17° C.; on 16.10.92, 15° C.
Temperature of refrigerator, 23.7.92, 9° C.; 20.10.92, 15° C.

* For explanation of the system of naming the flasks containing the experimental waters, see Note, p. 185.

unfiltered water. This greater impregnation of the unfiltered water was intentionally effected so as to heighten the contrast which it was anticipated would be presented at the close of the experiments. Thus on June 16, 1892, or 83 days from infection, the sterile waters still yielded several thousand anthrax colonies per cubic centimetre, whilst in the unfiltered water on the following day (June 17, 1892) only 1200 anthrax colonies could at most be detected. Or, again, comparing the results obtained by the special method of preliminary heating, on June 17, 1892, the unfiltered water yielded a maximum of 49 colonies per cubic centimetre; whilst on July 11, 1892, or nearly a month later, the porcelain-filtered water yielded by the same method 128—145 colonies per cubic centimetre, and the steamed water on the same day (July 11, 1892), 163—179 colonies per cubic centimetre.

There is thus again the most convincing proof that the degeneration of the spores of anthrax is more rapid in the unsterilised than in the sterilised water, whilst it is almost immaterial in this respect whether the latter is sterilised by steam or by filtration through porous porcelain, although there is some slight evidence that the steam-sterilised water is more favourable to the preservation of the anthrax germs than that which has been rendered sterile by filtration through porcelain.

As regards the influence of temperature on the preservation of anthrax, in the First Series of experiments the evidence apparently pointed in the direction of the degeneration taking place more rapidly at the summer than at the winter temperature; whilst in the Second Series of experiments the indications are uncertain, and I am of opinion, therefore, that the difference of temperature in question is probably a matter of little consequence in this respect.

Finally examining the results obtained in the last plate cultivations made in October, it will be seen that the anthrax germs were still present in the sterile waters (steamed and porcelain-filtered) in practically undiminished numbers, whilst in the unsterilised water their numbers were so much reduced that they could only just be still recognised by the special method of preliminary heating.

I made the following experiments in order to test the virulence of these infected sterile waters of the Second Series:—

Animal Experiment No. 6—On October 12, 1892, 1 c.c. of water from the flask "1 I, Thames water, steam-sterilised (Second Series), infected with anthrax on March 25, 1892," was subcutaneously injected into a white mouse. The mouse died within 2 days 21 hours; the body exhibited much œdema; the spleen was very much enlarged, and anthrax bacilli were fairly abundant in it, the gelatine cultures also developing the characteristic growths in due course.

Animal Experiment No. 7.—On October 12, 1892, 1 c.c. of water from the flask "2 I, Thames water, porcelain-filtered (Second Series), infected with anthrax, March 25, 1892," was subcutaneously injected into a white mouse. The mouse died within 2 days 5 hours; the body exhibited much œdema, but the spleen was very small; anthrax bacilli were found in the latter, both with the microscope and by cultivation in gelatine.

From these experiments it is evident, therefore, that *the sterile Thames waters (Second Series) were still virulent nearly 7 months after their infection with anthrax, and the virulence as measured by the rapidity of their lethal effect was noticeably greater than with the corresponding waters of the First Series.*

III. Experiments on the Vitality and Virulence of the *Bacillus anthracis* and its Spores in Loch Katrine Water.

Experiments were made on the same lines with the water supplied to Glasgow from Loch Katrine.

The water was collected personally by myself, on July 6, 1892, at the Anderson's College, Glasgow; some of the water was drawn directly into sterile bottles, and submitted to ordinary plate cultivation within a few hours of its collection. The plates, after three days' incubation at 18—20° C., yielded 74 colonies per 1 c.c. of water.

As this is, as far as I am aware, the first record of the plate cultivation of such moorland water, I take this opportunity of also referring to the results obtained by me, for another purpose, in the plate cultivation of a number of samples of that portion of the Dundee water supply which is derived from the Loch of Lintrathen, a very similar source to Loch Katrine. All the samples were taken during the months of June and July, and one in October, during the present year, and were submitted to cultivation in about one hour from the time of collection. The results were as follows:—

Date of collection.	No. of days the plates were incubated at 18—20° C.	No. of colonies obtained from 1 c.c. of water.
22.6.92	4	110
23.6.92	4	149
30.6.92	3	290
2.7.92	3	94
4.7.92	3	114
11.7.92	3	279
17.7.92	3	177
21.7.92	3	77
29.7.92	3	155
18.10.92	3	260

Table XI.—Behaviour of Bacteria normally present in Loch Katrine Water. Third Series of Experiments. Unfiltered Loch Katrine Water.

Date on which plates were poured.	Particular flask employed. *	Number of plate.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies from 1 c.c. of water. Total.	Number of colonies from 1 c.c. of water. Anthrax.	Remarks.
				c.c.			
8.7.92	..	341	4	$\frac{4}{11}$	512	0	The Loch Katrine water, collected 6.7.92, yielded on the day of collection, 74 colonies per 1 c.c.; this water remained in stoppered bottles at about 12° C. until 8.7.92, when these experiments were begun.
		342	5	$\frac{1}{11}$	803	0	
		345	7	$\frac{2}{25}$	900	0	
		346	7	$\frac{1}{25}$	925	0	
12.7.92	1 I	371	2	$\frac{3}{11}$	30,700	0	
	1 I	372	2	$\frac{1}{11}$	44,970	0	
	1 I	375	2	$\frac{2}{25}$	44,980	0	
	1 I	376	2	$\frac{1}{25}$	49,500	0	
	1 R	373	2	$\frac{1}{4}$	13,800	0	
	1 R	374	2	$\frac{1}{12}$	13,248	0	
	1 R	377	2	$\frac{2}{25}$	14,600	0	
	1 R	378	2	$\frac{1}{25}$	16,250	0	
22.7.92	1 I	408	3	3	4	0	3 c.c. water + 1 c c. broth heated for 2 min. at 70° C.
	1 I	409	3	2	5	0	2 c.c. ,, ,, ,, ,,
	1 R	410	2	3	39	0	3 c.c. ,, ,, ,, ,,
	1 R	411	2	2	62	0	2 c.c. ,, ,, ,, ,,
26.10.92	1 I	502	3	$\frac{1}{3}$	339	0	
	1 I	503	3	$\frac{1}{6}$	342	0	
	1 I	506	4	$\frac{2}{25}$	310	0	
	1 I	507	4	$\frac{1}{25}$	325	0	
	1 R	504	3	$\frac{1}{3}$	2,400	0	
	1 R	505	3	$\frac{1}{12}$	2,712	0	
	1 R	508	3	$\frac{2}{25}$	3,075	0	
	1 R	509	3	$\frac{1}{25}$	3,125	0	

During the vacation the temperature of incubator on 22.7.92 was 19° C.; on 20.9.92, 17° C.; on 16.10.92, 15° C.
,, ,, ,, refrigerator on 23.7.92, 9° C.; on 20.10.92, 15° C.

* For explanation of the system of naming the flasks containing the experimental waters, see Note, p. 185.

It should be pointed out that neither the Loch Katrine nor the Lintrathen waters are submitted to filtration before delivery, and that these figures, therefore, indicate the bacterial life present in these waters as they come from the loch, excepting in so far as changes may occur in their passage through the mains.

The behaviour of these bacteria normally present in the Loch Katrine water, when the latter is kept at winter and summer temperatures respectively, was investigated, and the results are recorded in Table XI. From this it will be seen that in four days a very considerable multiplication had taken place, the increase in numbers being much greater in the case of the incubator (19° C.) flask than in that of the refrigerator (9° C.). After three months, however, the numbers in the incubator flask had fallen very much below those in the refrigerator flask, showing that the higher temperature leads to rapid multiplication followed by rapid decline, whilst at the lower temperature the increase and decrease are of a more gradual character.

As in the experiments on the behaviour of the anthrax bacilli in Loch Katrine water, the latter was used both in the natural condition and sterilised by filtration through porous porcelain; the water was also submitted to chemical analysis both in its natural state and after passage through the porcelain filter, with the following results :—

Results of Analysis expressed in Parts per 100,000.

	Loch Katrine water (unfiltered).	Loch Katrine water (filtered through porcelain).
Total solid matters	3·00	3·00
Organic carbon	0·195	0·220
,, nitrogen	0·015	0·030
Ammonia (free)..........	0	0·002
,, (albuminoid) ...	0·003	0·004
Oxygen consumed by organic matter, as measured by reduction of a solution of permanganate acting for three hours in the cold	0·116	0·140
Nitrogen as nitrates and nitrites................	trace	trace
Total combined nitrogen ..	0·015	0·032
Chlorine	0·6	0·65
Temporary hardness	0	0
Permanent ,, ...	0·8	0·8
Total ,, ...	0·8	0·8
Remarks	clear and palatable	clear.

These analyses show the Loch Katrine water experimented with

to be of its usual character; as far as mineral ingredients are concerned, it is but little removed from distilled water; it contains, however, just about the same proportion of organic matter as Thames water, although the smaller yield of albuminoid ammonia and the larger amount of oxygen absorbed from permanganate show this organic matter to be qualitatively different. In point of fact, the organic matter in Loch Katrine water is almost exclusively of peaty origin, whilst that in Thames water, coming as it does from land which is under high cultivation, is derived from a variety of sources, both vegetable and animal.

The Loch Katrine water was also experimented with in three different states: (a) *in the natural condition unsterilised*, (b) *sterilised by filtration through porous porcelain, and* (c) *sterilised by steam.*

The method of infection was similar to that already described for Thames water; the sporiferous anthrax bacilli were taken from an agar-agar cultivation of four days' age. Eight needle loops full of the surface growth were introduced into 50 c.c. of sterile water, and thoroughly mixed by violently shaking for 15 minutes; 1 c.c. of this attenuation was then employed for infecting 750 c.c. of each of the three waters.

The waters, after infection, were distributed in a number of small flasks, which were then disposed of as follows:—

Loch Katrine water, unfiltered .	3 flasks in	refrigerator (6—10° C.).
	3 "	incubator (18—20° C.).
Loch Katrine water, filtered through porcelain	3 "	refrigerator.
	3 "	incubator.
Loch Katrine water, steamed ..	3 "	refrigerator.
	3 "	incubator.

Gelatine plates were in each case poured on the day of infection, and also on several occasions subsequently, after the lapse of various intervals of time. The results of these examinations will be found in the following Tables XII, XIII, and XIV:—

Table XII.—Anthrax in Loch Katrine Water. Third Series of Experiments. Unfiltered Loch Katrine Water, Infected with Anthrax, July 8, 1892.

Date on which plates were poured.	Particular flask employed. *	Number of plate.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies from 1 c.c. of water.		Remarks.
					Total.	Anthrax.	
				c.c.			
8.7.92	1 I	339	5	$\frac{1}{3}$	6,330	5880	
	1 I	340	5	$\frac{1}{12}$	5,160	4560	
	1 I	343	5	$\frac{2}{25}$	5,500	4750	
	1 I	344	5	$\frac{1}{25}$	5,050	4650	
12.7.92	1 I	363	2	$\frac{1}{4}$	Too much liquefied	Anthrax too small to count	
	1 I	364	2	$\frac{1}{12}$	97,920	,,	
	1 I	367	2	$\frac{2}{25}$	97,150	,,	
	1 I	368	2	$\frac{1}{25}$	109,500	,,	
	1 R	365	2	$\frac{1}{4}$	26,400	,,	
	1 R	366	2	$\frac{1}{12}$	27,840	,,	
	1 R	369	2	$\frac{2}{25}$	22,050	,,	
	1 R	370	2	$\frac{1}{25}$	22,225	,,	
22.7.92	1 I	403	3	3	1,224	1224	3 c.c. water + 1 c.c. broth heated for 2 min. at 70° C.
	1 I	404	3	2	1,044	1044	2 c.c. ,, ,, ,, ,,
	1 R	405	2	3	3,432	3432	3 c.c. ,, ,, ,, ,,
	1 R	406	2	2	4,002	4002	2 c.c. ,, ,, ,, ,,

* For explanation of the system of naming the flasks containing the experimental waters, see Note, p. 185.

Table XII—*continued.*

Date on which plates were poured.	Particular flask employed. *	Number of plate.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies from 1 c.c. of water.		
					Total.	Anthrax.	
				c.c.			
18.10.92	1 I	469	5	3	0	0	3 c.c. water + 1 c.c. broth heated for 2 min. at 70° C.
	1 I	470	5 and 6	3	2	0	3 c.c. „ „ „ „
	1 I	473	4	$\frac{5}{11}$	90	0	
	1 I	474	4	$\frac{1}{11}$	77	0	
	2 I	475	6	$\frac{5}{12}$	7	0	
	2 I	476	5	$\frac{1}{12}$	96	0	
	1 R	471	5	3	325	325	Practically pure cultivation of anthrax. 3 c.c. water + 1 c.c. broth heated for 2 min. at 70° C.
	1 R	472	3 and 4	3	..	Numerous typical anthrax colonies visible, but not far enough advanced to count.	3 c.c. water + 1 c.c. broth heated for 2 min. at 70° C.
	1 R	477	3	$\frac{5}{14}$	Too much liquefied		
	1 R	478	3	$\frac{1}{14}$	1,862	..	Anthrax noticed (microsc.), but too small to count.
	2 R	479	3	$\frac{5}{12}$	1,654	..	„ „ „ „
	2 R	480	3 and 4	$\frac{1}{12}$	2,184	..	„ „ „ „

Diffused Daylight.

These Flasks were placed in Diffused Daylight on July 23, 1892.

8.10.92	3 I	424	7	3	14	0	3 c.c. water + 1 c.c. broth heated for 2 min. at 70° C.
	3 I	425	7	3	7	0	3 c.c. ,, ,, ,, ,,
12.10.92	3 I	464	5	$\frac{5}{12}$	24	0	
	3 I	465	5	$\frac{1}{12}$	0	0	
	3 R	466	6	$\frac{5}{12}$	4,140	3780	
	3 R	467	6	$\frac{1}{12}$	6,072	5400	
18.10.92	3 I	481	6	$\frac{5}{12}$	Too much softened		
	3 I	482	6	$\frac{1}{12}$	360	0	
	3 R	483	6	$\frac{5}{12}$	5,806	5746	
	3 R	484	6	$\frac{1}{12}$	5,472	5436	

During the vacation the temperature of incubator on 22.7.92 was 19° C.; on 20.9.92, 17° C.; on 16.10.92, 15° C.
,, ,, ,, refrigerator on 23.7.92 was 9° C.; on 20.10.92, 15° C.

* For explanation of the system of naming the flasks containing the experimental waters, see Note, p. 185.

Table XIII.—Anthrax in Loch Katrine Water. Third Series of Experiments. Porcelain-filtered Loch Katrine Water, Infected with Anthrax, July 8, 1892.

Date on which plates were poured.	Particular flask employed. *	Number of plate.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies from 1 c.c. of water.		Remarks.
					Total.	Anthrax.	
				c.c.			
8.7.92	1 I	333	5	$\frac{5}{7}$	4,385	4,385	
	1 I	334	5	$\frac{5}{14}$	4,200	4,200	
12.7.92	1 I	357*a*	5	$\frac{5}{6}$	10,368	10,368	Gelatine was very much softened, hence over-estimated.
	1 I	358*a*	5	$\frac{5}{12}$	7,680	7,680	
	1 R	361*a*	7	$\frac{10}{11}$	5,754	5,754	
	1 R	362*a*	4	$\frac{5}{11}$	5,358	5,358	One large mould on plate, and thus obliged to count earlier than others.
18.7.92	1 I	381	6	$\frac{10}{13}$	Uncountable	All anthrax	
	1 I	382	6	$\frac{5}{13}$	7,212	7,212	
	1 R	385	6	$\frac{10}{11}$	5,005	5,005	
	1 R	386	6	$\frac{5}{11}$	6,160	6,160	
8.10.92	1 R	427	6	3	3,332	3,332	Experiments to determine diminution in number of anthrax colonies. 3 c.c. water + 1 c.c. broth heated for 2 min. at 70° C.
	1 R	428	6	3	Too much softened to count	..	3 c.c. water + 1 c.c. broth heated for 2 min. at 70° C.
	1 R	429	6	$\frac{2}{3}$	8,364	8,364	
	1 R	430	6	$\frac{1}{5}$	8,400	8,400	

11.10.92	2 I	441	6	$\frac{5}{6}$	4,315	4,315	
	2 I	442	6	$\frac{5}{12}$	4,176	4,176	
	1 R	445	6	$\frac{10}{13}$	7,233	7,233	
	1 R	446	6	$\frac{5}{13}$	6,340	6,340	
12.10.92	1 I	450	4	$\frac{2}{3}$	3,860	3,860	
	1 I	451	4	$\frac{1}{3}$	2,928	2,928	
	2 R	454	4	$\frac{10}{13}$	6,737	6,737	
	2 R	455	4	$\frac{5}{13}$	6,279	6,279	
Diffused Daylight. Flasks placed in Diffused Daylight on July 23, 1892.							
12.10.92	3 I	458	7	$\frac{10}{11}$	6,575	6,575	
	3 I	459	7	$\frac{5}{11}$	5,866	5,866	
	3 R	462	7	$\frac{5}{6}$	11,088	11,088	
	3 R	463	7	$\frac{5}{12}$	9,662	9,662	

During the vacation the temperature of incubator on 22.7.92, 19° C.; on 20.9.92, 17° C.; on 16.10.92, 15° C.
" " " refrigerator on 23.7.92, 9° C.; on 20.10.92, 15° C.

* For explanation of the system of naming the flasks containing the experimental waters, see Note, p. 185.

Table XIV.—Anthrax in Loch Katrine Water. Third Series of Experiments. Steam-sterilised Loch Katrine Water, Infected with Anthrax, July 8, 1892.

Date on which plates were poured.	Particular flask employed. *	Number of plate.	Number of days plates were incubated.	Volume of water employed for plate cultivation.	Number of colonies from 1 c.c. of water.		Remarks.
					Total.	Anthrax.	
				c.c.			
8.7.92	1 I	331	5	$\frac{2}{3}$	4,868	4,868	
	1 I	332	5	$\frac{1}{3}$	5,133	5,133	
12.7.92	1 I	355*a*	7	$\frac{2}{3}$	19,521	19,521	All possibly over-estimated through gelatine being much softened by anthrax colonies.
	1 I	356*a*	7	$\frac{1}{3}$	23,004	23,004	
	1 R	359*a*	7	$\frac{5}{6}$	12,420	12,420	
	1 R	360*a*	7	$\frac{5}{12}$	10,282	10,282	
18.7.92	1 I	379	6	$\frac{2}{3}$	Uncountable	All anthrax	Too much softened to count.
	1 I	380	6	$\frac{1}{3}$	,,	,,	,, ,, ,,
	1 R	383	6	$\frac{10}{11}$	,,	,,	,, ,, ,,
	1 R	384	6	$\frac{5}{11}$	,,	,,	,, ,, ,,
11.10.92	1 I	439	4	$\frac{2}{3}$	13,359	13,359	
	1 I	440	4	$\frac{1}{3}$	10,950	10,950	
	2 R	443	4	$\frac{5}{6}$	13,840	13,840	
	2 R	444	4	$\frac{5}{12}$	15,016	15,016	
12.10.92	2 I	448	7	$\frac{5}{6}$	10,890	10,890	Largely contaminated, but only anthrax colonies counted.
	2 I	449	7	$\frac{5}{12}$	13,266	13,266	
	1 R	452	4	$\frac{5}{6}$	29,426	29,426	Largely contaminated, and probably over-estimated. Results only approximate. Impossible to accurately estimate anthrax colonies.
	1 R	453	4	$\frac{5}{12}$	26,827	26,827	

Diffused Daylight.

Flasks placed in Diffused Daylight on July 23, 1892.

12.10.92	3 I	456	7	$\frac{5}{6}$	16,632	16,632	
	3 I	457	7	$\frac{5}{12}$	16,742	16,742	
	3 R	460	7	$\frac{2}{3}$	24,064	24,064	
	3 R	461	7	$\frac{1}{3}$	21,120	21,120	

During the vacation the temperature of incubator on 22.7.92 was 19° C.; on 20.9.92, 17° C.; on 16.10.92, 15° C.
,, ,, ,, refrigerator on 23.7.92, 9° C.; on 20.10.92, 15° C.

* For explanation of the system of naming the flasks containing the experimental waters, see Note, p. 185.

Turning in the first instance to Table XII, in which the results for the unsterilised water are recorded, it will be seen from the plates poured on the day of infection (July 8, 1892) that the sporiferous anthrax bacilli had been introduced to the amount of about 5000 per cubic centimetre, the water bacteria being present to the number of about 600 per cubic centimetre only. From Tables XIII and XIV it will be seen that the sporiferous anthrax bacilli had been introduced into the sterile waters in also just the same numbers as into the unsterile water, viz., about 5000 per cubic centimetre.

In the case of the unsterilised water, there was no difficulty in counting the anthrax colonies on the plates poured on the day of infection (July 8, 1892); but already, four days afterwards, the number of water bacteria had so greatly increased that the plates could not be preserved long enough for the proper development of the anthrax colonies, although they could still be just recognised as minute dots with a low power of the microscope. The multiplication of the water bacteria was greatest in the flask which had been kept at 18—20° C., although it was also very considerable in the one which had been in the refrigerator at about 9° C.

A fortnight (July 22, 1892) after the day of infection, anthrax was easily discoverable in large numbers by means of the special method of preliminary heating to destroy the water bacteria, there being three or four times as many colonies on the plates from the refrigerator flask as on those from the flask which had been kept at 18—20° C. This difference becomes still further accentuated later on, for on examining those flasks which had been continuously at the temperature (19° C.) of the incubator up to October 18, 1892, it was found that no anthrax could be demonstrated, whilst in those flasks which had been at the temperature of the refrigerator (9° C.) up to July 23, 1892, and up to 15° C. afterwards, a large number of anthrax colonies was obtained on cultivation. This contrast was presented both by those flasks which were kept continuously in the dark, as well as by those which had been placed in the daylight from July 23, 1892, onwards. From this it would appear that the spores of anthrax undergo markedly more rapid degeneration in the unsterilised Loch Katrine water at 20° C. than at 9° C. As will be seen presently, this striking phenomenon is not exhibited by the sterilised Loch Katrine water, in which there is little difference between the numbers of anthrax colonies obtained from the incubator and refrigerator flasks respectively.

It became, of course, particularly interesting to ascertain whether these remarkable differences between the incubator and refrigerator flasks would be maintained also in respect of virulence, and to determine this point the following direct experiments were made :—

Animal Experiment No. 11.—On October 16, 1892, 1 c.c. of the

water from the flask "1 I, Loch Katrine, unfiltered, infected with anthrax, July 8, 1892," was subcutaneously injected into a white mouse. The mouse is still alive (November 10, 1892), or 25 days after the operation.

Animal Experiment No. 22.—On October 23, 1892, 1 c.c. of water from the flask "2 I, Loch Katrine, unfiltered, infected with anthrax, July 8, 1892," was subcutaneously injected into a white mouse. The mouse is still alive (November 10, 1892), or 18 days after the operation.

Animal Experiment No. 19.—On October 21, 1892, 1 c.c. of water from the flask "1 R, Loch Katrine, unfiltered, infected with anthrax, July 8, 1892," was subcutaneously injected into a white mouse. The mouse died within 2 days 15 hours; the body exhibited extensive œdema; the spleen was much enlarged, and was found full of anthrax bacilli, the characteristic growth being obtained in gelatine cultivation.

Animal Experiment No. 23.—On October 23, 1892, 1 c.c. of water from the flask "2 R, Loch Katrine, unfiltered, infected with anthrax, July 8, 1892," was subcutaneously injected into a white mouse. The mouse died within 2 days 16 hours, and, although no bacilli could be microscopically detected in the spleen, their presence was revealed by gelatine cultivations made from that organ, thus leaving no doubt that the animal succumbed to anthrax.

Thus, the experiments made on mice with the unsterilised Loch Katrine water are in precise harmony with the results obtained by plate cultivation; the two incubator flasks failed to kill, whilst the two corresponding refrigerator flasks were fatal to the mice, into which they were injected in the same quantity.

The same striking contrast was likewise obtained in the case of two similar flasks, which had, however, been exposed to daylight since July 23, 1892. Thus—

Animal Experiment No. 12.—On October 17, 1892, 1 c.c. of water from the flask "3 I, Loch Katrine, unfiltered, infected with anthrax, July 8, 1892, exposed to daylight since July 23, 1892," was subcutaneously injected into a white mouse. The mouse is still alive (November 10, 1892), or 18 days since the operation.

Animal Experiment No. 13.—On October 17, 1892, 1 c.c. of water from the flask "3 R, Loch Katrine, unfiltered, infected with anthrax, July 8, 1892, exposed to daylight since July 23, 1892," was subcutaneously injected into a white mouse. The mouse died within 2 days 18 hours; the body exhibited much œdema; the spleen was slightly enlarged, and anthrax bacilli were discovered in the latter, both by the microscope and by cultivation in gelatine.

Thus, in the case of the flasks subsequently exposed to daylight, also, the incubator flask proved harmless, and the refrigerator flask fatal, to the mice, into which they were respectively injected in equal quantity.

It was not, however, to be forthwith concluded, that the anthrax germs in these incubator flasks were necessarily extinct, and the endeavour was made, as in previous cases described above, to revive them, by the addition of broth to the waters. On October 29, 1892, therefore, 5 c.c. of sterile broth were accordingly added to each of the flasks "1 I," and "2 I," which were then placed in an incubator at 37° C., after which the following two experiments were performed:—

Animal Experiment No. 28.—On October 31, 1892, 0·5 c.c. of the water from the flask (to which broth had been added on October 29, 1892) "1 I, Loch Katrine, unfiltered, infected with anthrax, July 8, 1892," was subcutaneously injected into a white mouse. The mouse is still alive (November 14, 1892), or 14 days after the operation, and, therefore, out of danger of succumbing to anthrax.

Animal Experiment No. 29.—On October 31, 1892, 0·5 c.c. of the water from the flask (to which broth had been added on October 29, 1892) "2 I, Loch Katrine, unfiltered, infected with anthrax, July 8, 1892," was subcutaneously injected into a white mouse. The mouse died within 2 days 19 hours, anthrax bacilli being found in the spleen, and the characteristic growth obtained on gelatine cultivation.

Thus, in the case of flask "1 I," the anthrax germs were extinct and could not be revived with broth; in the case of "2 I," however, the addition of broth restored the virulence, so that some few anthrax germs must still have been alive in this flask.

Turning, in the next instance, to Table XIII, we find that on the day of infection (July 8, 1892) the porcelain-filtered Loch Katrine water yielded about 4000 colonies per cubic centimetre, whilst four days later (July 12, 1892) the number had risen to about 8000 in the flask kept at 18—20° C., whilst the refrigerator flask exhibited only a slight increase on the original number, a similar difference being observable again on the tenth day (July 18, 1892). From July 23, 1892, the temperature of the refrigerator was permitted to follow that of the room, and on October 8, 11, and 12, 1892, these refrigerator flasks yielded 6000—8000 anthrax colonies, whilst the incubator ones had fallen again to the original number of about 4000 per cubic centimetre.

Again, in the case of the flasks which had been exposed to the daylight from July 23, 1892, onwards, it was found, on October 12, 1892, that the refrigerator flask contained about 10,000, the incubator flask only 6000, anthrax germs.

It will be seen, therefore, that although there is evidence of the numbers being longer maintained at the low than at the high temperature, the contrast between the two is enormously less marked than in the case of the unsterilised Loch Katrine water.

In order to test the virulence of the porcelain-filtered water, I made the following experiments:—

Animal Experiment No. 15.—On October 17, 1892, 1 c.c. of water from the flask "3 I, Loch Katrine, porcelain-filtered, infected with anthrax, July 8, 1892, exposed to daylight since July 23, 1892," was subcutaneously injected into a white mouse. The mouse died within 2 days 17 hours; the body exhibited much œdema; the spleen was slightly enlarged, and anthrax bacilli were found in it, both by the microscope and by gelatine cultivation.

The porcelain-filtered Loch Katrine water was, therefore, fully virulent more than three months after infection with anthrax.

Again, if we turn to Table XIV, in which the results with the steam-sterilised Loch Katrine water are recorded, we find that the multiplication after four days is more pronounced than in Table XIII, and, again, it will be seen that the multiplication is markedly greater in the flask kept at 18—20° C. than in the one kept in the refrigerator, although the latter also shows a multiplication of 100 per cent. In the later examinations, after the temperature of the refrigerator had been allowed to rise to that of the room (about 15° C.), the numbers in the incubator and refrigerator flasks became more equalised; on the whole, the numbers were, at the final examination, greater in the refrigerator than in the incubator flasks, and this is markedly the case also in those flasks which, from July 23, 1892, onwards, were exposed to diffused daylight.

To test the virulence of the steam-sterilised Loch Katrine water, the following experiment was made:—

Animal Experiment No. 14.—1 c.c. of water from the flask "3 I, Loch Katrine, steam-sterilised, infected with anthrax, July 8, 1892, exposed to daylight since July 23, 1892," was subcutaneously injected into a mouse. The mouse died within 1 day 20 hours; the body exhibited extensive œdema; the spleen was very much enlarged, and was found to be full of anthrax bacilli, which yielded the characteristic growth in gelatine cultivation.

Thus, the steam-sterilised Loch Katrine water was highly virulent more than three months after being infected with anthrax.

Conclusions to Part I.

The results obtained by me in the course of the above investigation on the vitality and virulence of anthrax spores in potable water may be summarised in the following statements:—

1. Three distinct series of experiments were made, viz.:—

Series I, in which Thames water collected above Staines was used, and a very small number of anthrax germs introduced into the water (pp. 181—213).

Series II, in which the same water was employed, but a much larger number of anthrax germs introduced (pp. 213—225).

Series III, in which the water of Loch Katrine, taken as typical of a moorland supply, was employed, and a large number of anthrax germs introduced (pp. 225—239).

2. In all these three series of experiments the waters were infected with anthrax from an agar-agar cultivation of such age as to ensure the abundant presence of spores, so that the investigation deals exclusively with the vitality and virulence of sporiferous anthrax bacilli (pp. 184, 213, 228).

3. *In the sterilised waters both of the Thames and Loch Katrine* the sporiferous anthrax bacilli maintain themselves in practically undiminished numbers for long periods of time—many months. In nearly all cases, moreover, a distinct increase in the numbers was in the first instance observed, which was followed in those cases in which the experiments were extended over a sufficiently prolonged period by a decline, which, however, in no case resulted in less than about one half of the original number of anthrax spores being left in the water after seven months. In the sterile Loch Katrine waters the anthrax spores after upwards of three months were still two or three times as numerous as in the first instance; indeed, there was more distinct evidence of multiplication in the sterile Loch Katrine waters than in either of the two series of Thames water experiments (pp. 200—204, 219—225, 232—235, 238, 239).

As far as any difference could be established between the behaviour of the anthrax spores in these sterile waters at winter (4—10° C.) and summer (18° C.) temperatures respectively, the balance of evidence was on the whole in favour of the numbers being longer maintained at the low than at the high temperature, although the preliminary increase took place more rapidly at the high temperature and was earlier followed by the subsequent decline (pp. 204, 224, 238).

Practically no difference could be established between the behaviour of the anthrax germs in water *sterilised by steam* and by *filtration through porous porcelain* respectively; in the case of the Loch Katrine water there was indeed some evidence of the steam-sterilised water being more favourable to the anthrax spores than that which had been filtered through porcelain (pp. 201—203, 220—223, 232—234).

No effect could be traced to the influence of *diffused daylight* on the behaviour of the anthrax spores in these sterile waters, the numbers in daylight and in darkness being practically the same (pp. 204—206, 208, 221, 223, 233, 235).

On the other hand, *direct sunshine* exerted a most marked effect,

for after 56 hours' insolation the number of anthrax spores was greatly diminished, and after 84 hours' exposure to solar radiation the presence of anthrax was no longer demonstrable by cultivation at all (pp. 209—213).

As regards the *virulence of the anthrax* in the sterilised waters of the Thames and Loch Katrine, the experiments which I have performed on mice conclusively prove that this is maintained over long periods of time—many months. In no single instance did the injection of 1 c.c. of these waters fail to kill the mouse, although the anthrax spores had been in the Thames water for upwards of seven months, and for upwards of three months in that of Loch Katrine. There is, however, unmistakable evidence of the rapidity of the lethal action of the anthrax depending on the number of spores present in the water. Thus in the First Series of Thames water experiments, in which only a small number of anthrax spores were present, the porcelain-filtered water was fatal in 4 days 17 hours, the steam-sterilised water which had been exposed to daylight was also fatal in 4 days 17 hours, and the porcelain-filtered water similarly exposed to daylight killed the mouse in 6 days $20\frac{1}{2}$ hours; on the other hand, in the Second Series of Thames water experiments, in which a much larger number of anthrax spores were present in the water, the porcelain-filtered was fatal in 2 days 5 hours, and the steam-sterilised in 2 days 21 hours; and again in the case of the Loch Katrine water, in which a still larger number of anthrax spores were present at the time of the experiment, the porcelain-filtered killed in 2 days 17 hours, and the steam-sterilised in 1 day 20 hours (pp. 204—206, 224, 225, 239).

Of the sterilised Thames waters exposed to *direct sunshine*, neither the porcelain-filtered nor the steam-sterilised was fatal to mice, nor could their virulence be revived by the addition of broth to the water (pp. 209, 212).

4. *In the unsterilised Thames water*, both of the First and Second Series of experiments, the anthrax spores were indeed found to be still present in a vital state after many months, but in greatly diminished numbers, and thus furnishing the most striking contrast to their behaviour in the same water when sterilised either by steam or by porcelain filtration.

In the unsterilised Thames water of the First Series of experiments, anthrax was only just discoverable by the special method of cultivation which I devised for the purpose (see p. 185) 4 months after infection, so that it must have undergone great diminution in numbers during this period (pp. 195, 196, 198, 199).

This diminution in the number of anthrax spores was further established by the experiments on animals. Thus, when mice were subcutaneously injected with 1 c.c. of these unsterilised waters of the First Series, 7 months after the anthrax spores had been introduced,

the mice were not killed. That some few living anthrax spores were, however, still present in the water was proved by adding some broth to the water, which led to such a multiplication of the anthrax, that in the course of a few days the water thus treated became fatal on injection into mice. It is worthy of note in this connexion that one only of the unsterilised Thames waters (First Series) proved fatal without broth being added, and that this water had been filtered through Swedish paper before its infection with anthrax, so that it approached to some extent in its character the sterilised waters (pp. 193—194, 197, 200).

In the case of the unsterilised Thames water again it appears, as far as the evidence goes, that the anthrax spores are better preserved in the water at the winter than at the summer temperature (pp. 193, 197).

Daylight again appeared to be slightly unfavourable to the preservation of anthrax, the indications in this direction being more marked in the case of the First than in the Second Series of Thames water experiments (pp. 205, 218).

Sunlight, on the other hand, was most pronounced in its deleterious effect on the anthrax spores in the unsterilised Thames water. In the unfiltered water they were no longer discoverable by cultivation after 84 hours' sunshine, whilst in the paper-filtered water there were still a few present after 92 hours, but all had disappeared after 151 hours' insolation. These waters also which had been thus exposed to direct sunshine proved innocuous to mice, nor could their virulence be resuscitated by the addition of broth, clearly showing that the anthrax spores had perished to the last individual (pp. 209—212).

It is especially noteworthy in connexion with these results that they establish the remarkable fact that the anthrax spores, when immersed in water, are less prejudically affected by sunlight than when immersed in any of the ordinary culture materials. Thus, it has been shown by a number of observers that the anthrax spores suspended in broth and other culture materials are generally destroyed in the course of a few hours' exposure to sunshine, whilst in the above experiments the anthrax spores immersed in Thames water, both sterile and unsterile, resisted an insolation of upwards of 56 hours. This remarkable contrast between the behaviour of the anthrax spores in an aqueous and a nutrient medium respectively is also in accordance with the previous observations of Straus and of Momont, who both, however, appear to have experimented with distilled water only (pp. 212, 213).

In the unsterilised Thames water experiments of the Second Series, the conditions were different, inasmuch as the water was in the first instance infected with a much larger number of sporiferous anthrax bacilli. On this account, although a great diminution in the number

had taken place during the seven months' residence in the water, yet sufficient remained even then to be discoverable by cultivation, and to prove fatal to mice when 1 c.c. of the water was subcutaneously injected into them. Only in one instance did a mouse remain alive after receiving such an injection, and on repeating the experiment, the second mouse injected with the same water duly died of anthrax (pp. 214—219).

5. *In the unsterilised Loch Katrine water*, the behaviour of the anthrax spores was particularly remarkable. At the commencement of the experiment there were about 5000 anthrax germs and 500 other micro-organisms per cubic centimetre. These micro-organisms underwent, as was to be anticipated, very large multiplication, especially in that portion of the water which was kept at a summer temperature. Fourteen days after the commencement of the experiment cultivation still showed the anthrax to be abundant, but their number was markedly greater in that portion of the water which had been kept at winter than in that kept at summer temperature (p. 229).

On re-examination 3 months after the beginning of the experiment, the anthrax was absolutely undiscoverable by cultivation in the water kept at summer temperature, whilst it was still present in considerable, although greatly diminished numbers, in the water which had been kept at the lower temperature (p. 230).

The same difference was observed ir respect of virulence also, for in every case mice injected with the low temperature water died of anthrax, whilst those which received the same quantity of the higher temperature water remained alive. Indeed it was not until broth was added to this water kept at summer temperature that, of two flasks so treated, the one became virulent, whilst the other still remained innocuous (pp. 236—238).

It should be mentioned also that in this Loch Katrine water kept at the higher temperature (18° C.) the ordinary water bacteria became very much diminished in number after the preliminary multiplication referred to above (pp. 226, 227).

This remarkable bactericidal power of the unsterilised Loch Katrine water kept at 18° C. is doubtless due to the elaboration by the water bacteria of toxic products from the peaty organic matter present in this water, which products cause the destruction either of the spores or of the bacilli into which the anthrax spores will at this temperature gradually germinate. Moreover, the difference in this respect between the Thames water and that of Loch Katrine is to be sought for in the different nature of the organic matter present in these waters. The analyses show that quantitatively the organic matter (as measured by organic carbon and nitrogen) in both waters is almost exactly the same, but qualitatively they are very different, that in the Loch Katrine water being much less oxidised than that in Thames

water, as measured by the oxygen which they respectively absorb from permanganate. This doubtless means that the Loch Katrine organic matter has hitherto been a comparative stranger to bacterial life, whilst the organic matter of the Thames has been more fully exploited by the micro-organisms which are more abundant in its waters. It is evident, however, that the Loch Katrine organic matter *per se* is not possessed of bactericidal powers at the higher temperature, for in the sterilised Loch Katrine waters at that temperature the anthrax spores underwent no such destruction (pp. 182, 227, 232—235, 238).

Part II.

"Experimental Investigations on the Behaviour of *Bacillus anthracis* in Water." By Professor Marshall Ward, D.Sc., F.R.S., assisted by G. E. Cartwright Wood, M.D., B.Sc.*

It is obvious that some of the questions raised in our Report can only be settled by experimenting directly with the freshly collected water, and, since we selected a definite type of Thames water for our work, it was necessary to determine the main points in the natural history of this water itself, and to employ it directly for cultures.

Some of the principal results are submitted as follows:—

Bacteriological Examination of the Thames Water in its Natural Condition.

Preliminary.

On January 21, 1892, three samples of Thames water were submitted to examination immediately after collection, to obtain an answer to the question, Does the Thames water selected for inquiry contain bacteria at the moment of collection?

Plate cultures were made in the usual way, by dropping known quantities, so many drops from a pipette containing 1 c.c., and known to emit so many drops per 1 c.c., of the water into gelatine melted at 30° C.

Sample I gave the following results:—

(*a.*) 1-drop plates incubated 5 days at 15° C. (drop = $\frac{1}{23}$ c.c.) gave an average of 2 colonies per plate = 46 bacteria per 1 c.c.

(*b.*) 3-drop plates in 7 days gave an average of 8 colonies per plate = 61 bacteria per 1 c.c.

(*c.*) 9-drop plates in 5 days gave an average of 8 colonies per plate = 10 bacteria per 1 c.c.

So far, it was clear that the river water contains *some* bacteria, 10 to 61 per c.c., which develop on gelatine. These were of several kinds, and developed at different rates, and pure cultures of the different forms were isolated for future reference, as it was part of our object to familiarise ourselves with the normal bacterial flora of the river.

Samples II and III were similarly examined, and with similar results, which need not be detailed here.

* All experiments on animals have been made by Dr. Cartwright Wood.

SERIES A.

Table A.—Thames Water in Natural State—Cultures made forthwith.

Dates on which water was collected.	Date of making plate.	Condition of water.	Number of days incubated.	Temperature, degrees C.	Number of drops used for plate.	Number of drops per 1 c.c.	Actual number of colonies on plate.	Calculated total number of bacteria per 1 c.c.	Remarks.
21.3.92	21.3.92	Fresh from river and untreated.	3	20°—22	1	24	29	696	
,,	,,	,,	3	,,	1	25	39	975	
,,	,,	,,	3	,,	3	24	104	832	
,,	,,	,,	3	,,	3	22	98	719	The plates liquefied so rapidly, it was impossible to count the numerous colonies accurately.
,,	,,	,,	4	12—15	6	25	about 200	?	
,,	,,	,,	4	,,	9	22	?	?	
20.6.92	20.6.22	,,	3	20	1	35	7	245	
,,	,,	,,	3	,,	6	30	100	500	
10.12.92	10.12.92	,,	2	20	2	25	69	862	Many liquefying.
,,	,,	,,	,,	,,	4	25	200	1250	
,,	,,	,,	,,	,,	9	25	390	1083	

In all cases the Thames water experimented with contains an appreciable, but not necessarily large, number of living bacteria, capable of germination and growth on gelatine plates. As will be seen later on, the above numbers are very small, no doubt owing to the low temperature of the water, and the promptness of the cultures direct from the river; moreover, the number per 1 c.c., as shown by gelatine plate-cultures, is to a great extent dependent on the temperature of incubations.

Series A.

In this series of experiments I confined my attention to the numbers of bacteria actually present in the Thames water when collected. The method followed was the usual one of carefully collecting the samples in sterile flasks, or occasionally in sterilised vacuum tubes drawn to a point which is broken under the water by forceps: these being heated and the glass point re-sealed in a spirit lamp at the river itself.

In no case given was the water allowed to stand more than a couple of hours or so, and then at low temperatures, and in some cases (employed as checks on the others) the plates were actually made within half an hour of collecting.

The method of making the plates was also the well-known one, and does not need description here; I employed round or square Petri's dishes in all cases.

Examples illustrating the results of these examinations are given in Table A.

Table A shows very clearly that *the number of bacteria actually present in the Thames water at the moment of collecting is not large*, for an open river, though differences appear to exist in June as contrasted with March and December as regards the numbers. I do not propose to go into these differences at present, however, since they are not striking, but it is worth noting that the experience of previous observers favours the supposition that monthly differences in the total number of bacteria of rivers are to be expected.*

Of course the point could only be decided by continuous observation, which I think should be carried out. It is, perhaps, not without interest to note that, so far as my few observations on this point go, they bear out the conclusions of Miquel,† that there are more bacteria in the river in March than in June, and more in December than in either of these months, a fact probably correlated with the surface drainage and rain washings. I repeat, however, that

* See, for instance, Miquel, 'Manuel pratique d'Analyse Bactériologique des Eaux,' 1891, pp. 128—146, and the literature on rivers in our 1st Report.

† *Loc. cit.*, pp. 131—133.

my observations on this particular point are only by the way, and much too few for any valuable conclusions on the wider question.

As regards the species or forms met with, I defer their discussion for the present; suffice it to say that we carefully isolated and tabulated the different forms in order to experiment with them afterwards (see p. 285), and that a considerable number of the individuals (not species) were rapidly liquefying forms, rendering the keeping of the plates difficult.

Series B.

This series, part of a more extensive set of experiments to be referred to later, is calculated to show the kind of changes as regards the number of normal water bacteria, undergone by the river-water on standing. It should be regarded, therefore, as supplementing the results in the last table.

In each case the same procedure was adopted, and of course the same care in collecting the samples and making the plates, &c.

The collected water was placed in properly sterilised and plugged flasks, and allowed to stand, at the temperature given, undisturbed for a number of days.

Plates were made forthwith to determine the initial numbers of bacteria per 1 c.c. of the water, and then samples taken every twenty-four or forty-eight hours, for several days, to determine whether any, or what, increase or decrease in the total numbers had occurred in the interval.

It was to be expected, from the publications of others and from our own experience, that such increase would occur, and my preliminary experiments showed that in this case the increase is very great. Consequently we had to take precautions against having our plates too over-crowded with colonies, and this we did by adding pure distilled water to each sample taken for analysis in such quantities that the bacteria in 1 c.c. of the original water were distributed through 20 c.c., and making the plates from the diluted sample.

It is true this method involves the risk of killing some of the bacteria; but the results show that the numbers obtained are very large, nevertheless.

It is also true that the dilution method introduces a further source of error in compelling us to multiply the ascertained result—always a dangerous process in statistics. However, we have been unable to see any way out of this difficulty, and have relied rather on the general results expressed in the tables than on the actual numbers, which it is evident must be looked upon as approximations only.

Series B.

Table B (I).—Thames Water in Natural State. Cultures made forthwith and after standing.

Date of collecting water.	Date of making plate.	Condition of water.	Number of days incubated.	Temperature, C.	Quantity used for plate.	*Diluted or not.	Number of colonies counted on plate.	Calculated total number of bacteria per 1 c.c.	Remarks.
12.5.92	12.5.92	fresh from river	3	18°—20	c.c. $\frac{1}{25}$	not	6	175	
”	”	”	3	”	$\frac{6}{25}$	”	55	225	
”	13.5.92	standing 24 hours at 20° C.	3	”	$\frac{1}{5}$	diluted 20 times	1200 to 1600	120,000 to 360,000	100 squares contained (averaged by counting several carefully in different parts of the field) about 12 to 16 colonies each. Maximum and minimum chosen.
”	”	”	3	”	$\frac{3}{5}$	”	4800	160,000	The $\frac{1}{4}$ plate covered 20 squares, averaging 60 colonies per square.
”	14.5.92	standing 48 hours at 20°	2	”	$\frac{1}{5}$	”	2160 to 3240	216,000 to 324,000	18 squares per $\frac{1}{4}$ plate gave from 30 to 45 colonies per square.
”	”	”	2	”	$\frac{3}{5}$	”	4800 to 5800	160,000 to 193,333	15 squares per $\frac{1}{4}$ plate gave 80 to 90 colonies per square.
”	16.5.92	standing 96 hours at 20°	2	”	$\frac{1}{5}$	”	2560 to 3200	256,000 to 320,000	16 squares per $\frac{1}{4}$ plate = 40 to 50 colonies each.
”	”	”	2	”	$\frac{2}{5}$	”	3200 to 3840	160,000 to 192,000	16 squares per $\frac{1}{4}$ plate = 50 to 60 colonies per square.
”	18.5.92	standing 144 hours at 20°	2	”	$\frac{1}{5}$	”	150	15,000	
”	”	”	2	”	$\frac{2}{5}$	”	200	10,000	

* Diluted with sterile distilled water.

Series B.

Table B (II).—Thames Water in Natural State. Cultures made forthwith and after standing.

Date of collecting water.	Date of making plate.	Condition of water.	Number of days incubated.	Temperature, C.	Quantity used for plate.	*Diluted or not.	Number of colonies counted on plate.	Calculated total number of bacteria per 1 c.c.	Remarks.
20.6.92	20.6.92	fresh from river	3	18°—20	c.c. $\frac{1}{35}$	not	7	245	Used 1 drop from a pipette which = 35 drops per 1 c.c.
"	"	"	3	"	$\frac{1}{5}$	"	100	500	
"	21.6.92	standing 24 hours at 18—20° C.	3	"	$\frac{1}{5}$	diluted 20 times	125	12,500	
"	"	"	3	"	$\frac{3}{5}$	"	280	9,333	
"	22.6.92	standing 48 hours at 18—20° C.	3	"	$\frac{1}{5}$	"	4200	420,000	In this case we found that 60 squares averaged 70 colonies each.
"	"	"	3	"	$\frac{3}{5}$	"	6000	200,000	100 squares averaged 60 colonies each.
"	23.6.92	standing 72 hours at 18—20° C.	2	"	$\frac{1}{5}$	"	7000 to 8000	700,000 to 800,000	100 squares averaged 70 to 80 colonies each.
"	"	"	2	"	$\frac{3}{5}$	"	uncountable	..	Partly owing to the enormous number, and partly to liquefaction, it was impossible to estimate.
"	24.6.92	standing 96 hours at 18—20° C.	1	"	$\frac{1}{5}$	"	100,000 ?	1,000,000?	100 squares contained an average of 100 colonies each.
"	25.6.92	standing 120 hours at 18—20° C.	3	"	$\frac{1}{5}$	"	7000 to 9000	700,000 to 900,000	100 squares contained an average of 70 to 90 colonies each.

* Diluted with sterile distilled water.

If we take the average numbers in the ninth column of Tables B (I) and B (II), it is clear that *a very rapid rise in the numbers occurred during the first twenty-four hours, and continues during the second day, and even to the third or fifth, and then comes a fall, slight at first, and then rapid.* Now, without insisting too closely on the numbers—indeed, we expressly desire to emphasize the fact that they can only be more or less approximate, from the nature of the case—it is interesting to note how closely the general result compares with the experience of other observers, working with the waters of rivers, &c., in other parts of the world. For the sake of this *general* comparison, I append our own averages and those of one or two other workers in the annexed tabular *résumé*, where the averages are taken in round numbers as approximations.

Numbers of Bacteria per 1 c.c. of Water.

Source of water.	Number of hours the water had stood at about 20° C.										
	0—2	24.	48.	72.	96.	120.	144.	10 days.	20 days.	30 days.	
Thames....	200	200,000	445,000	..	232,000	..	12,500	..	..	..	In May (Table B, I).
,, ...	372	10,400	300,000	750,000	1,000 000	800,000	..	..	..	..	In June (Table B, II).
,, ...	1065	83,300	337,500	12,500	18,100	8,250	..	225	..	..	In December.
Varne.....	125	38,000	125,000	590,000	..	..	..	..	..	..	Miquel, *loc. cit.*, p. 14.
,, ...	71	71,000	..	..	1,070,000	..	..	..	..	..	
Well water.	1990	*18,660	..	†26,100	..	‡37,000	..	..	§4,700	..	Meade Bolton, 'Zeit. f. Hyg.,' 1886, p. 76.
,,	143	12,457	..	328,543	..	..	..	‖233,452	¶17,436	..	Cramer. See Hueppe, 'Schilling's Journ.,' 1887, p. 43.

* Is given as 24—36 hours.
§ Given as 20—30 days.
† Given as 2—4 days.
‖ Given as 8th day.
‡ Given as 5—10 days.
¶ = 17th day.

Experiments to test the Influence of Temperature on the Changes undergone by the Normal Bacteria of Standing Thames Water.

To determine whether the effect of temperature on the increase of the bacteria of standing Thames water is very marked, the experiments summarised in Table *x* were carried out.

Two 1-litre flasks of the water were collected and at once carefully analysed, with the result that the Thames water collected on December 10 contained, on the average, 1065 bacteria per 1 c.c.

The flasks were then placed in the dark, one at 12° C., the other at 20° C., and examined periodically, with the results tabulated.

The column of remarks sufficiently denotes the behaviour noticed.

Table *x*.—Changes undergone by Thames Water on standing at 12° C. and at 20° C. in December.

Number of days flask stood.	Temperature at which flask stood.	Date of making plate.	Number of days plate was incubated at 20° C.	Quantity of water used for plate.	Diluted or not.	Number of colonies found on plate.	Calculated number of bacteria per 1 c.c. original.	Remarks.
	° C.			c.c.				
0	..	Dec. 10	2	$\frac{2}{25}$	Not	69	862	Average 1065, of which 331 were already evident as liquefying forms.
0	..	"	2	$\frac{4}{25}$	"	200	1,250	
0	..	"	2	$\frac{9}{25}$	"	390	1,083	
1	20	Dec. 11	2	$\frac{1}{25}$	"	5,000	125,000	Average 83,333, of which about 100 were already liquefying badly.
1	"	"	2	$\frac{3}{25}$	"	5,000	41,666	
1	12	"	2	$\frac{1}{15}$	"	70	1,750	Average 2858, of which 40 were badly liquefying forms.
1	"	"	2	$\frac{3}{25}$	"	500	4,166	
2	20	Dec. 12	2	$\frac{1}{25}$	"	17,500	437,500	Average 337,500, of which very many were badly liquefying.
2	"	"	2	$\frac{2}{25}$	"	19,000	237,500	
2	12	"	2	$\frac{3}{25}$	"	1,950	12,916	Average 14,999 — many badly liquefying.
2	"	"	2	$\frac{3}{25}$	"	2,050	17,083	
3	20	Dec. 13	2	$\frac{1}{25}$	"	..	..	Plate spoilt by over-heating, therefore must accept the second plate as average, though there also the colonies were retarded by over-heating the plate.
3	"	"	2	$\frac{1}{25}$	"	510	12,500	
3	12	"	2	$\frac{1}{25}$	"	24,000	600,000	Average 675,000, of which a large proportion liquefy.
3	"	"	2	$\frac{1}{25}$	"	30,000	750,000	
4	20	Dec. 14	2	$\frac{2}{25}$	"	310	23,250	Average 18,187, of which only about 500 are distinctly liquefying.
4	"	"	2	$\frac{2}{25}$	"	175	13,125	
4	12	"	2	$\frac{2}{25}$	"	5,500	412,500	Average 656,250, of which at least 15,000 are liquefying.
4	"	"	2	$\frac{2}{25}$	"	12,000	900,000	
5	20	Dec. 15	2	$\frac{1}{25}$	"	40	6,000	Average 8250, the liquefying forms much fewer.
5	"	"	2	$\frac{1}{25}$	"	70	10,500	

5	12	,,	2	$\frac{1}{25}$	,,	600	90,000	Average 60,000, with 1500 liquefying forms.
5	,,	,,	2	$\frac{1}{25}$	,,	200	30,000	
7	20	Dec. 17	2	$\frac{1}{25}$	,,	29	725	Of which 100 liquefied.
7	12	,,	2	$\frac{1}{25}$	,,	850	21,250	Of which 500 liquefied.
8	20	Dec. 18	2	$\frac{1}{25}$	,,	6	150	None liquefying.
8	12	,,	2	$\frac{1}{25}$	,,	450	11,250	Of which 500 liquefy.
10	20	Dec. 20	2	$\frac{3}{25}$	,,	27	225	Of which 25 liquefy.
10	12	,,	2	$\frac{1}{25}$	,,	196	4,900	Of which 375 liquefy.
13	20	Dec. 23	2	$\frac{1}{25}$	,,	..	..	Spoilt by moulds.
13	12	,,	2	$\frac{3}{25}$	,,	62	456	Of which 25 liquefy.
18	20	Dec. 28	3	$\frac{3}{25}$	,,	105	875	N.B.—Only five or six showed on second day ; the temperature rose to 25° C.
18	12	,,	2	$\frac{3}{25}$	,,	236	1,966	

The results seem to show conclusively that *the maximum number is not only higher at the higher temperature, but that it is attained more rapidly.* That this is, at least in part, due to the bacteria being enabled to multiply and diffuse themselves through the liquid more rapidly, before the available oxygen and food materials are diminished, seems an obvious conclusion; though I do not believe that these factors alone explain the phenomenon.

Experiments with the Vegetative Bacilli *of Anthrax in Thames Water.*

It is necessary to know, if possible, whether the living vegetative bacilli of anthrax can survive immersion in such waters as we have experimented with, and then to see if they can multiply therein: that spores can withstand such immersion has long been known, and we gave very full particulars on this point in our First Report,* but the evidence regarding the vegetative bacilli is somewhat conflicting, and consequently I have devoted attention especially to this point. The difficulties are decidedly great. In the first place it is not easy to obtain spore-free bacilli, and it will be objected that in some of the following cases it is not certain that my material was absolutely spore-free; this cannot be gainsaid, but it can at least be claimed from the experiments that, while they do not absolutely settle the question whether the vegetative bacilli can or cannot multiply in Thames water, they do show that, if such bacilli obtain access to the water and form spores in it, they are very tenacious of life and difficult to exterminate.

Preliminary.

On January 28, 1892, a sterilised $\frac{1}{2}$-litre flask was charged with 25 c.c. of Thames water, fresh from the river, and inoculated with a large charge of a potato cultivation of a normal anthrax grown at 16° C., and devoid of spores, so far as could be ascertained. We employed a potato culture in order to introduce as little nitrogenous food material as possible into the water, and chose a tube grown at a relatively low temperature (16° C.) to try and prevent the precocious development of spores.

Five $\frac{1}{2}$-litre flasks were then charged each with 25 c.c. of the freshly-collected Thames water, and inoculated each with 1 c.c. of the above infecting fluid. The flasks were marked A, B, C, D, and E.

Flask A was selected for periodic examination, to obtain a preliminary answer to the question, Can *Bacillus anthracis* maintain itself alive at all in Thames water? Pipettes were selected to drop 25 drops to the 1 c.c.

After standing 24 hours at 20° C., we made a series of plates (on

* See 'Roy. Soc. Proc.,' vol. 51, 1892, pp. 219 and 268.

January 29) with 1, 3, and 9 drops each respectively, and examined next day. The annexed example is selected.

1-drop plate	= 5 colonies	= 125 per 1 c.c.	*No anthrax.*
3 ,,	= 67 ,,	= 558 ,,	,,
9 ,,	= 260 ,,	= 720 ,,	,,

This seemed to show that the vegetative bacilli rapidly disappear from the water, a result apparently in accordance with the experience of several previous observers.

A liquefying bacillus was common on the plates, however, and prevented our keeping them long enough to determine whether anthrax was really absent, or merely slower in development than the rest of the organisms.

A new set of plates were made from Flask A on January 30, *i.e.*, the flask having stood 48 hours at 20° C.

1-drop plate = 107 colonies = 2675 per 1 c.c.,

while plates with 3 and 9 drops respectively liquefied so rapidly that we could make no determinations of the numbers.

No anthrax colonies were found to develop in the time, and similar results were obtained next day, the flask having then stood 72 hours

The 1-drop plate = 320 colonies = 7900 per 1 c.c.

On February 2, the flask having stood 110 hours, a further set of plates were prepared, but the colonies developed were so numerous that we could not estimate them. On some of the plates, however, very small anthrax colonies appeared, and even in relatively large numbers. On the whole, this preliminary examination convinced us that such plates may fail to show anthrax colonies, because the normal water bacteria present develop so rapidly, and in such abundance, that the anthrax has no chance, especially if bad liquefying forms are present. They also showed us that the water forms increase in numbers, day by day, as the water stands.

As our further experiments show, *the conclusion that the anthrax died in these flasks is quite unwarranted; its persistence was due to the formation of spores;* but at the stage here reached that was a question to be inquired into.

Series B′.

This series was also designed to see whether virulent normal anthrax was capable of living as bacilli, and multiplying in the Thames water, either untouched or rendered sterile by filtration through porcelain or by heat, or if it passes over into spores in the water. In this series we boiled the Thames water for two hours.

The anthrax employed was a very virulent one, and known as "Edinburgh Cow A," grown on agar for thirty hours at 30° C. Eight flasks were used, and divided into four pairs; each flask received 25 c.c. of the water to be examined, and a large charge of anthrax—1 c.c. of the infecting fluid, which contained chiefly, if not entirely, bacilli.

Two flasks were charged with the Thames water in its crude state, and not infected at all.

Two were charged similarly with the crude water forthwith infected with anthrax.

Two were charged with the Thames water forthwith filtered through porcelain, and at once infected.

Two were charged with the boiled Thames water infected at once on cooling.

All stood at 20° C. in the dark.

In order to meet any such objection as that the original water possibly contained the spores of anthrax, we proceeded as follows:—

On June 27, the flasks having stood for seven days, we took samples of the original raw (non-infected) Thames water, and heated them at 60° C. for twenty-four hours. Plates made from this gave no signs of anthrax colonies. We also inoculated a guinea-pig with 1 c.c. of the raw water (not sterilised) injected into the peritoneum; this animal lived uninjured, whereas a guinea-pig inoculated with 1 c.c. of the raw water infected with anthrax died in thirty-six hours, and cover glass preparations and cultures made from the organs proved that it died normally of anthrax.

Table *a.*—Thames Water infected forthwith with *Virulent* Anthrax.

Date on which water was collected.	Date of making plate.	Number of days water stood at 20° C.	Number of days plate was incubated at 20° C.	Quantity of water used for plate.	Diluted or not.	Number of colonies on plate.		Calculated average number of bacteria per 1 c.c. of water.		Remarks.
						Total.	Anthrax.	Total.	Anthrax.	
June 20	June 20	0	3	c.c. $\frac{1}{5}$	* $\frac{1}{10}$th dilution	2,750	2744	137,500	137,200	Only 6 water colonies on the plate; all the rest = anthrax.
,,	,, 20	0	3	$\frac{3}{5}$	,,	7,200	7180	120,000	119,600	20 colonies on plate = water form.
,,	,, 21	1	3	$\frac{1}{5}$	$\frac{1}{20}$th dilution	600	12	60,000	1,200	
,,	,, 21	1	3	$\frac{3}{5}$	,,	740	7	74,000	700	
,,	,, 22	2	3	$\frac{1}{5}$	,,	10,000	?	1,000,000	?	A few very small and much retarded anthrax colonies.
,,	,, 22	2	3	$\frac{3}{5}$	,,	Too many to count	?	?	?	Probably over 1,000,000, but could neither count nor recognise anthrax; probably very few.
,,	,, 23	3	1	$\frac{1}{5}$	,,	5,000	Some	500,000	?	We examined microscopically while the colonies were very small. Anthrax was present, but entirely swamped a few hours later by the water forms.
,,	,, 24	4	2	$\frac{1}{5}$	,,	10,000	Some	1,000,000	?	A few anthrax colonies still there.
,,	,, 25	5	2	$\frac{1}{5}$	,,	..	..	..	..	Liquefied and spoilt.

* *i.e.*, 1 : 9 of infecting fluid and sterile water respectively.

Table *a* shows very clearly how the anthrax rapidly falls in quantity during the first three days, whereas the normal aquatic flora takes the lead and runs through the usual phases of rapid rise to a maximum and then an eventual fall. I have not included their further behaviour here, however, because it was impossible to trace the anthrax any longer on the plates.

On June 27, we heated a sample of the water—having then stood seven days at 20° C.—at 60° C. for twenty-four hours, and made a series of plates from it. With the exception of one or two water organisms on one of the plates, we obtained beautifully pure cultures of anthrax, proving beyond doubt that spores had been introduced or formed in the flasks. This was confirmed by inoculating a guinea-pig.

Table *b*.—Thames Water filtered forthwith through Porcelain and infected with Virulent Anthrax.

Date on which water was collected.	Date of making plate.	Number of days water stood at 20° C.	Number of days plate was incubated at 20° C.	Quantity of water used for plate.	Diluted or not.	Number of colonies on plate.		Calculated average numbers of bacteria per 1 c.c. of water.		Remarks.
						Total.	Anthrax.	Total.	Anthrax.	
June 20	June 20	0	3	c.c. $\frac{1}{5}$	$\frac{1}{10}$th dilution	3000	3000	150,000	150,000	
„	„ 20	0	3	$\frac{3}{5}$	„	6000	6000	100,000	100,000	
„	„ 21	1	3	$\frac{1}{5}$	$\frac{1}{20}$th dilution	1350	1350	135,000	135,000	
„	„ 21	1	3	$\frac{3}{5}$	„	2700	2700	90,000	90,000	
„	„ 22	2	3	$\frac{1}{5}$	„	1250	1250	125,000	125,000	
„	„ 22	2	3	$\frac{3}{5}$	„	1380	1380	46,000	46,000	
„	„ 23	3	3	$\frac{1}{5}$	„	1050	1050	105,000	105,000	
„	„ 24	4	3	$\frac{1}{5}$	„	1250	1250	125,000	125,000	
„	„ 25	5	3	$\frac{1}{5}$	„	750	750	75,000	75,000	

Table *c*.—Thames Water boiled for two hours and forthwith infected with *Virulent* Anthrax.

Date on which water was collected.	Date of making plate.	Number of days water stood at 20° C.	Number of days plate was incubated at 20° C.	Quantity of water used for plate.	Diluted or not.	Number of colonies on plates.		Calculated average numbers of bacteria per 1 c.c. of water.		Remarks.
						Total.	Anthrax.	Total.	Anthrax.	
June 20	June 20	0	3	c.c. $\frac{1}{5}$	$\frac{1}{10}$th dilution	1650	1650	82,500	82,500	
,,	,, 20	0	3	$\frac{3}{5}$	,,	2100	2100	35,000	35,000	
,,	,, 21	1	3	$\frac{1}{5}$	$\frac{1}{20}$th dilution	280	280	28,000	28,000	Grew very slowly; we could not determine why.
,,	,, 21	1	3	$\frac{3}{5}$	,,	1650	1650	55,000	55,000	
,,	,, 22	2	3	$\frac{1}{5}$	,,	750	750	75,000	75,000	
,,	,, 22	2	3	$\frac{3}{5}$	,,	900	900	30,000	30,000	Again a remarkable retardation of growth. We cannot explain it unless it is due to all the colonies arising now from *spores*.
,,	,, 23	3	3	$\frac{1}{5}$	,,	..	..	..	..	Culture spoilt and lost by an accident.
,,	,, 23	3	3	$\frac{3}{5}$	,,	1250	1250	43,000	43,000	
,,	,, 24	4	3	$\frac{1}{5}$	,,	660	660	66,000	66,000	
,,	,, 25	5	3	$\frac{1}{5}$	,,	1500	1500	150,000	150,000	

If we compare Table *a* with Tables *b* and *c*, there is a striking contrast as regards the maintenance of the anthrax colonies on the plates. This is no doubt largely due to the removal of the water organisms, enabling us to count the anthrax colonies so much more readily; but I do not believe it is solely due to that cause. It seemed much more likely—and a comparison of these tables with those of Series C appeared to bear out the probability—that the competition of the water organisms really affects the anthrax more directly, partly owing to the former taking what organic food materials there are, and so starving the anthrax, and partly owing to the rapid de-oxygenation of the water by the competing forms. As will be shown later, these normal water bacteria are aërobic in a very high degree, as we have convinced ourselves by actual experiments; and we have been surprised, therefore, at these results in the raw Thames water. As will be seen in the sequel, however, the behaviour of the organisms towards one another cannot be predicted (see pp. 290—298).

There is one point in connection with the *boiled* Thames water cultures (Table *c*) which seems worth further investigation: it is the remarkable retardation of growth exhibited on some of the plates after the first twenty-four hours. It seems by no means unlikely that the explanation is due to two causes :—

(1) The boiled water has been so far de-oxygenated that the *living* bacilli fall off, and only those which can pass into the spore condition maintain themselves, and as it takes longer to get cultures from the spores than from the actively vegetating bacilli, this might well explain the retardation seen on the plates.

(2) It may also be, however, that boiling the water renders many of the organic food substances less available for the growth of anthrax, and thus a partial starvation concurs in the fall.

Or (3) it may be due simply to osmotic phenomena consequent on immersion in the water.

In any case it seems worth while to note the apparently more rapid fall in the numbers in the *boiled* as contrasted with the *filtered* water, in the first forty-eight hours, though we think the matter would need a special inquiry to make certain of the phenomenon.

Series C.

This series of experiments was designed to secure answers to the following questions :—Do the bacilli of the anthrax live and multiply in Thames water at all, apart from any persistence of the spores? If so, is there any difference in their behaviour in the crude water, taken fresh from the river, with all its normal bacteria flora and other impurities, and in the same water deprived of the aquatic microbes by filtration through porcelain, or sterilised by boiling? And, further,

does the behaviour of strong virulent anthrax, known to be capable of producing vigorous spores, differ from that of weak or "attenuated" anthrax, known to be less deadly to animals, though still capable of forming spores if the right conditions are offered, in any respects, and in any or all of the waters?

The virulent anthrax employed was obtained from Edinburgh (and recorded as Cow No. 3), and was proved to be fatal to rabbits in two days); the attenuated anthrax also came from Edinburgh (Cow No. 1), and took five days to kill a rabbit.

The experiments detailed in the following tables, C (I) to C (VI), were arranged as follows:—

I. Four flasks, two of a litre capacity, and two of half a litre each, were filled with the crude Thames water, brought fresh from the river, and infected forthwith with anthrax: one pair of flasks receiving strong anthrax, the other weak anthrax: plate cultures were made at once, and on each succeeding day, the flasks standing in an incubator at 20—22° C., the whole time.

II. Four similar flasks were filled with the Thames water, same collection, filtered forthwith through porcelain (Chamberland filter), and proved to be free from aquatic bacteria, and duplicate pairs treated in exactly the same way.

III. Four similar flasks were filled with the Thames water, same collection, and treated exactly as before, excepting that the water was sterilised by heating in a steam steriliser to 100° C. for two hours.

IV. Finally, four similar flasks were filled with the raw Thames water, exactly as in set I, excepting that no anthrax was added, as we wished to determine by daily plate culture how the water organisms of the normal water behaved apart from the anthrax.

As regards the incubation and future care, &c., all the 16 flasks were treated alike, and the conditions of comparison are, therefore, the same.

The infecting fluid was obtained as follows in each case:—Clean sowings were taken from an active agar culture, then shaken up with sterile distilled water, and some of the dilute sowing spread on potato (in tubes) and incubated for 24 hours at 30° C.

This gave vigorous vegetative cultures, free from spores, as we satisfied ourselves by placing samples at 60° C. for 18 hours, and then making plate cultures, and we then proceeded as follows:—

The potato cultures were broken down in sterile distilled water, care being taken to introduce as little potato as possible, and charges of this placed in the flasks. Of each pair of flasks infected, one received four times as much as the other; the charge is referred to in the tables as "large," or "small," accordingly. It may here be stated that in those cases where experience showed us that large numbers of colonies were to be expected on the plates, we used

not only small doses of the liquid to be tested, *e.g.*, 1 or 3 drops from a pipette discharging 25 drops to the 1 c.c., but also diluted the liquid with sterile distilled water, *e.g.*, 1 : 4, or 1 : 9, &c., facts which we bring out duly in the tables.

To those critics who would remark on the dangers of the "dilution method" above referred to, I would reply in two ways: (1) it is the only practicable method available for getting over the difficulty of plates so densely crowded with colonies that no attempt at counting (or estimating) is possible; and (2) it certainly does not lead to *exaggeration* of the numbers of colonies, but in the contrary direction, and, therefore, the final numbers obtained are more likely to be below than above the truth.

As a matter of experience, I am, in fact, more and more assured that the whole procedure of gelatine plate cultivation leads to under-estimation rather than to over-estimation, and this is obviously a fault on the better side of exactness, since we have to be content with approximations. Nevertheless, great care has to be taken in all stages of manipulation, and the more so because it is always necessary to multiply out the final results.

The results of the daily examination of the non-infected flasks show the usual rise to a maximum, and then fall in the numbers of normal aquatic organisms existing in the Thames water. I now pass to the results obtained with weak and strong anthrax respectively, in the crude Thames water, *i.e.*, without filtering or sterilising in any way, Tables C (I) and C (II).

Series C. Table *c* (I).—Crude Thames Water infected forthwith with *Strong* Anthrax.

Date on which water was collected.	Date of making plate.	Number of days water stood at 20° C.	Number of days plate was incubated at 20°C.	Charge of anthrax used.	Quantity of water used for plate.	Diluted or not.	Number of colonies on plate.		Calculated average numbers of bacteria per 1 c.c. of water.		Remarks.
							Total.	Anthrax.	Total.	Anthrax.	
					c.c.						
Mar. 22	Mar. 22	0	3	Large	$\frac{1}{25}$	Not	40	20	1000	500	The anthrax colonies were abundant, and about as many as the water forms, but liquefaction and crowding out made it impossible to count accurately.
,,	,, 22	0	3	,,	$\frac{3}{25}$	,,	Too many to count	Most were anthrax	?	?	23 colonies of organisms were found and innumerable anthrax. This would give 2300 water forms per 1 c.c., and anthrax in much higher proportion.
,,	,, 23	1	3	,,	$\frac{1}{25}$	$\frac{1}{5}$th dilution	?	?	?	?*	Plate liquefied, but numerous anthrax colonies seen.
,,	,, 23	1	3	,,	$\frac{6}{25}$	,,	?	?	?	?	Ditto.
,,	,, 24	2	2	Small	$\frac{1}{25}$	,,	288	12	144,000	6,000	
,,	,, 24	2	2	,,	$\frac{3}{25}$	,,	1200	800	200,000	133,300	
,,	,, 25	3	3	,,	$\frac{1}{25}$	$\frac{1}{10}$th dilution	58	0	58,000	0	No anthrax visible on keeping another day.
,,	,, 25	3	3	,,	$\frac{3}{25}$	,,	130	?	43,300	?	No colonies of anthrax visible on this day, but next day we detected a few.
,,	,, 26	4	3	,,	$\frac{1}{25}$	,,	26	1	26,000	1,000	
,,	,, 26	4	3	,,	$\frac{3}{25}$	,,	80	?	23,300	?	Here, again, a few feeble anthrax colonies were observable next day, evidently being crowded out.
,,	,, 29	7	3	Large	$\frac{1}{25}$	,,	130	35	32,500	8,750	
,,	,, 29	7	3	,,	$\frac{3}{25}$	,,	300	?	25,000	?	Doubtful if any anthrax.

* In order to be sure if anthrax was present a mouse was inoculated, with positive results.

SERIES C. Table *c* (II).—Crude Thames Water infected forthwith with *Weak* Anthrax.

Date on which water was collected.	Date of making plate.	Number of days water stood at 20° C.	Number of days plate was incubated at 20° C.	Charge of anthrax used.	Quantity of water used for plate.	Diluted or not.	Number of colonies on plate.		Calculated average number of bacteria per 1 c.c. of water.		Remarks.
							Total.	Anthrax.	Total.	Anthrax.	
					c.c.						
Mar. 22	Mar. 22	0	3	Large	$\frac{1}{25}$	Not	Innumerable	Innumerable	?	?	There were 4 water organisms on plate, which gives 100 per 1 c.c. and countless anthrax.
,,	,, 22	0	3	,,	$\frac{3}{25}$	,,	,,	,,	?	?	27 water colonies on plate = 225 per c.c. and innumerable anthrax.
,,	,, 23	1	1	,,	$\frac{1}{25}$	$\frac{1}{5}$th dilution	?	?	?	?	Liquefied in 24 hours; anthrax was present, however.
,,	,, 23	1	1	,,	$\frac{6}{25}$	,,	?	?	?	?	,, ,, ,,*
,,	,, 24	2	2	Small	$\frac{1}{25}$	,,	508	11	204,000	5,500	
,,	,, 24	2	2	,,	$\frac{3}{25}$	,,	1160	?	193,300	?	Could not estimate the anthrax.
,,	,, 25	3	4	,,	$\frac{1}{25}$	$\frac{1}{10}$th dilution	130	3	130,000	3,000	The anthrax did not appear till 4th day; probably many suppressed.
,,	,, 25	3	4	,,	$\frac{3}{25}$	,,	221	35	73,600	11,600	
,,	,, 26	4	3	,,	$\frac{1}{25}$	,,	23	10	23,000	10,000	
,,	,, 26	4	3	,,	$\frac{3}{25}$	,,	85	25	28,300	8,300	
,,	,, 29	7	3	,,	$\frac{1}{25}$	,,	200	35	200,000	35,000	
,,	,, 29	7	3	,,	$\frac{3}{25}$	,,	350	Many	116,600	?	Could not estimate the anthrax, but very many colonies there.

* A mouse received 5 drops subcutaneously.

Series C. Table *c* (III).—Thames Water filtered through Porcelain, and forthwith infected with *Strong* Anthrax.

Date on which water was collected.	Date of making plate.	Number of days water stood at 20° C.	Number of days plate was incubated at 20° C.	Charge of anthrax used.	Quantity of water used for plate.	Diluted or not.	Number of colonies on plate.		Calculated average number of bacteria per 1 c.c. of water.		Remarks.
							Total.	Anthrax.	Total.	Anthrax.	
Mar. 22	Mar. 22	0	3	Large	c.c. $\frac{1}{25}$	Not	4000 to 6000 ?	4000 to 6000 ?	100,000 to 150,000 ?	100,000 to 150,000 ?	Pure anthrax.
"	" 22	0	3	"	$\frac{3}{25}$	"					Too many to count; certainly not fewer than in last, and pure anthrax.
"	" 23	1	3	"	$\frac{1}{25}$	"	3000 to 5000 ?	3000 to 5000 ?	75,000 to 125,000 ?	75,000 to 125,000 ?	All anthrax.
"	" 24	2	5	Small	$\frac{1}{25}$	$\frac{1}{5}$th dilution					Many anthrax, and a few intruded forms; temperature had been allowed to fall, and development was too slow.
"	" 25	3	6	"	$\frac{1}{25}$	$\frac{1}{10}$th dilution	480	480	480,000	480 000	Very clean pure culture.
"	" 25	3	6	"	$\frac{3}{25}$	"	1040	1040	346,600	346,600	Too many to count, and a small intruder was present, still very numerous anthrax, and on the 3rd day the culture seemed pure anthrax.
"	" 29	7	6	Large	$\frac{1}{25}$	"	?	?	?	?	
"	" 29	7	6	"	$\frac{3}{25}$	"	Too many to count	Pure anthrax	Too many to estimate	Pure anthrax.	

SERIES C.

Table *c* (IV).—Thames Water filtered through Porcelain and forthwith infected with *Weak* Anthrax.

Date on which water was collected.	Date of making plates.	Number of days water stood at 20° C.	Number of days plate was incubated at 20° C.	Charge of anthrax used.	Quantity of water used for plate.	Diluted or not.	Number of colonies on plate.		Calculated average number of bacteria per 1 c.c. of water.		Remarks.
							Total.	Anthrax.	Total.	Anthrax.	
					c.c.						
Mar. 22	Mar. 22	0	3	Large	$\frac{1}{25}$	Not	600	600	15,000	15,000	
,,	,, 22	0	3	,,	$\frac{3}{25}$	,,	3500	3500	29,100	29,100	
,,	,, 23	1	4	,,	$\frac{1}{25}$	,,	2500	2500	62,500	62,500	
,,	,, 23	1	4	,,	$\frac{3}{25}$	,,	4500	4500	37,500	37,500	
,,	,, 24	2	3	Small	$\frac{1}{25}$	$\frac{1}{5}$th dilution	850	?	425,000	?	A very large proportion was anthrax, but a foreign form had intruded into the flask.
,,	,, 24	2	3	,,	$\frac{3}{25}$	,,	Too many to count	All anthrax	?	?	
,,	,, 25	3	3	,,	$\frac{1}{25}$	$\frac{1}{10}$th dilution	?	?	?	?	Plate spoilt, but some anthrax was recognised.
,,	,, 29	7	6	Large	$\frac{1}{25}$	,,	4800	4800	1,200,000	1,200,000	
,,	,, 29	7	6	,,	$\frac{3}{25}$	,,	Too many to count	?	?	?	All pure anthrax, and certainly not fewer than last.

SERIES C. Table *c* (V).—Thames Water sterilised forthwith by heat and infected with *Strong* Anthrax.

Date on which water was collected.	Date of making plate.	Number of days water stood at 20° C.	Number of days plate was incubated at 30° C.	Charge of anthrax used.	Quantity of water used for plate.	Diluted or not.	Number of colonies on plate.		Calculated average number of bacteria per 1 c.c. of water.		Remarks.
							Total.	Anthrax.	Total.	Anthrax.	
					c.c.						
Mar. 22	Mar. 22	0	3	Large	$\frac{1}{25}$	Not	5000	5000	125,000	125,000	
,,	,, 22	0	3	,,	$\frac{3}{25}$	,,	Too many to count	Too many to count	?	?	Pure anthrax. Not fewer than in last case.
,,	,, 23	1	3	,,	$\frac{1}{25}$	,,	5000 to 6000	5000 to 6000	125,000 to 150,000	125,000 to 150,000	
,,	,, 23	1	3	,,	$\frac{3}{25}$	,,	Too many to count	Too many to count	?	?	As above; not fewer than before.
,,	,, 24	2	5	Small	$\frac{1}{25}$	$\frac{1}{5}$th dilution	1000	1000	500,000	500,000	One or two yellow colonies were not anthrax.
,,	,, 24	2	5	,,	$\frac{3}{25}$	,,	?	?	?	?	About same number as last.
,,	,, 25	3	6	,,	$\frac{1}{25}$	$\frac{1}{10}$th dilution	2400	2400	2,400,000	2,400,000	Nothing visible on 4th day.
,,	,, 25	3	6	,,	$\frac{3}{25}$	,,	3600	3600	1,200,000	1,200,000	
,,	,, 29	7	6	Large	$\frac{1}{25}$	,,	1200	1200	300,000	300,000	
,,	,, 29	7	6	,,	$\frac{3}{25}$	,,	1200	1200	100,000	100,000	

SERIES C.

Table *c* (VI).—Thames Water sterilised forthwith by heat and infected with *Weak* Anthrax.

Date on which water was collected.	Date of making plate.	Number of days water stood at 20° C.	Number of days plate was incubated.	Charge of anthrax used.	Quantity of water used for plate.	Diluted or not.	Number of colonies on plate.		Calculated average number of bacteria per 1 c.c. of water.		Remarks.
							Total.	Anthrax.	Total.	Anthrax.	
Mar. 22	Mar. 22	0	3	Large	c.c. $\frac{1}{25}$	Not	2500	2500	62,500	62,500	
,,	,, 22	0	3	,,	$\frac{3}{25}$	,,	Too many to count	?	75,000	?	Pure anthrax. Too numerous to estimate, but not fewer than last.
,,	,, 23	1	3	,,	$\frac{1}{25}$	,,	3000	3000	?	75,000	
,,	,, 23	1	3	,,	$\frac{8}{25}$	,,	Too many to count	?	275,000	?	As above.
,,	,, 24	2	3	Small	$\frac{1}{25}$	$\frac{1}{5}$th dilution	550	550	?	275,000	
,,	,, 24	2	3	,,	$\frac{3}{25}$	,,	2000	2000	333,300	333,300	
,,	,, 25	3	6	,,	$\frac{1}{25}$	$\frac{1}{10}$th dilution	2000	2000	2,000,000	2,000,000	
,,	,, 25	3	6	,,	$\frac{3}{25}$	,,	4400	4400	1,466,600	1,466,600	
,,	,, 29	7	6	Large	$\frac{1}{25}$	,,	2500	?	625,000	625,000	Not quite pure; a few yellow colonies had intruded.
,,	,, 29	7	6	,,	$\frac{3}{25}$	,,	Too many to count	?	?	?	Pure anthrax, but far too numerous to estimate.

Here we see, on examining Table C (I), in spite of unavoidable imperfections in the observations, due to the difficulties of counting and of observing when liquefaction commences, that both the anthrax and the water organisms may run a similar course as regards the first few days; in both cases the climax is rapidly reached (about the third day) and then a decline sets in. But it is worth notice that even after seven days' standing the anthrax is not eliminated, and we were so struck with the importance of this phenomenon that I decided to employ further tests to see if this persistence was really due to the continued vegetation of the anthrax or to the development of spores.

I was driven to suspect spores by several facts. In the first place Strauss and Dubarry have shown* that anthrax *can* form spores after being placed in water, provided the temperature is not too low (20° C.); secondly, we noted in some plates that the anthrax colonies were hanging back, so to speak, in their development, and it seemed not unlikely that this was due to time being needed for the germination of spores.

To test this point we placed a few cubic centimetres from one of the flasks of this group on April 7, *i.e.*, eight days after the last culture, for 24 hours at 60° C., and, before heating the liquid, inoculated a guinea-pig and a mouse with a trace of it.

Both guinea-pig and mouse were dead on April 9, *i.e.*, after 48 hours, and that their death was due to anthrax was proved by finding the bacilli in the blood of the heart, and by obtaining pure cultures therefrom.

The water heated to 60° C. for 24 hours gave pure cultures of anthrax also, showing conclusively that spores had been formed in the water. These cultures also justify the conclusion that aquatic normal forms did *not* develop spores, unless we assume that their spores are less resistent to moderately high temperatures. Without laying too much stress on the numbers, therefore, I think Table C (I) shows that while *Bacillus anthracis* can only live vegetatively and maintain its hold for about three days in the crude Thames water at 20° C., it can form spores there which enable it to live for a longer period,† and I conclude *not* that the competing water forms destroy the bacilli, but *that the decrease of anthrax on the plates is due partly to its passing into the spore condition, and to the*

* See our First Report, p. 268.

† We shall show later on that these spores can remain alive for several months, a result well established by previous observers. Duclaux, for instance, found that there were spores still alive in some of Pasteur's old flasks which had been kept for twenty-one or twenty-two years, and showed that in those flasks where they had died it was probably owing to the acid or alkaline reaction of the media. (See De Bary, 'Lectures on Bacteria,' 1887, p. 54.)

aquatic forms developing so rapidly, and some of them so quickly liquefying the gelatine, that even when plenty of anthrax exists on the plates the latter are rendered useless before they can be got to develop visible colonies.

On comparing Tables C (III) and C (IV) the result comes out that both weak and strong anthrax can hold their own for some time in the filtered Thames water, and that this is not a mere case of their lying passive and unchanged in it; indeed, without laying undue stress on the actual numbers, the general result *seems* to be that this schizomycete multiplies vegetatively under the conditions given, and then passes over into spores. I say this *seems* to be the case; but it is much more likely that the apparent increase at first is due to *the breaking up of the bacilli into shorter rodlets, most of which die at last.*

That spores were really present we proved, as before, by submitting samples of each of the waters to 60° C. for 24 hours, and then cultivating plates from them; the beautifully pure cultures of anthrax obtained showed clearly that spores had been formed.

I are aware of the criticism that the vegetative growth exhibited by both the weak and strong anthrax was probably not entirely at the expense of the organic materials already in the filtered water, but was no doubt in part due to small quantities of food materials introduced with the infecting material (and possibly in part also due to substances derived from decomposing bacteria); but the reply is (1) that the quantity of food materials introduced by our mode of infection was very small, and (2) that it does not affect the practical question much, because in nature such minimum fouling of the water would be likely to occur when anthrax finds its way to the river. Of course the criticism should be borne in mind, however, and our experimental results do not support the idea that anthrax can multiply vegetatively in waters containing only minimum traces of food materials.

If we compare Tables *c* (V) and *c* (VI) the fact again appears to come out that the anthrax bacillus, whether strong or weak as regards its virulence, behaves very like an ordinary water form when first placed in Thames water sterilised by steam. Here, again, the explanation given above no doubt applies. Moreover, it is again evident that, as time goes on, the plates need more incubating to bring out the bacilli, and the numbers are then very large—*cf.* the events of the third day—a fact which again raised our suspicions as to the development of spores. As before, moreover, we tested this suspicion by heating a sample of each water to 60° C. for twenty-four hours, and obtained pure cultures of anthrax therefrom.

Series D.

This series of experiments was designed to seek answers to the following questions:—1. Can an anthrax known to be incapable of developing spores (asporogenous) maintain itself in the Thames water side by side with other organisms, or in the same deprived of the water forms by filtering through porcelain?* 2. Is there any appreciable difference in behaviour between the asporogenous anthrax and a virulent race known to be capable of developing spores?

As matters turned out, we had to abandon the series in the middle, owing to the discovery that our so-called "asporogene" was a very much enfeebled form, but not utterly devoid of the sporogenous power,† and partly owing to a mishap with the filtered series. We select some of the results—see Tables D (I) to D (III)—because the approximate numbers obtained are useful; but we are engaged in repeating the series with a more reliable culture of the "asporogenous anthrax."

The arrangement of the flasks, &c., was much as before. Four flasks were filled with the crude Thames water, not infected, and examined daily. Four flasks were filled with the same collection of water, and to these anthrax was added as follows:—Two received virulent anthrax liquid in the proportion of 1 c.c. to every 25 c.c. of water; and two received the same proportion of liquid through which "asporogene" anthrax was distributed.

Finally four similar flasks were filled with the water (same batch), filtered forthwith through a Chamberland porcelain filter, and infected exactly as the last. The virulent anthrax came from an agar culture, growing well at 30° C. for 24 hours, and in excellent condition. The "asporogene" anthrax was also growing vigorously on agar under the same conditions. In each case the infecting liquid was made by evenly distributing quantities of the anthrax, as equal as possible in quantity and as free from agar as we could remove it from the tubes, in sterile distilled water. It will be noticed that all the plates were made with a dilution of 20 times as much water as corresponded to the original infected liquid. As before, the plates were made daily for a week, and the numbers given in the columns depend on several countings.

* We decided not to use the water sterilised by heat in this series, in order to reduce the number of flasks and plates which it would entail.

† The asporogenous anthrax employed was not the spontaneous natural form, but one produced artificially by degeneration with carbolic acid.

SERIES D.

Table D (I).—Crude Thames Water infected forthwith with *Virulent* Anthrax.

Date on which water was collected.	Date of making plate.	Number of days water stood at 20° C.	Number of days plate was incubated at 20° C.	Quantity of water used for plate.	Diluted or not.	Number of colonies on plate.		Calculated average number of bacteria per 1 c.c. of water.		Remarks.
						Total.	Anthrax.	Total.	Anthrax.	
				c.c.						
May 12	May 12	0	7	$\frac{1}{5}$	$\frac{1}{20}$th dilution	4	0	400	0	No anthrax appeared.
"	" 12	0	7	$\frac{3}{5}$	"	792	780	26,400	26,000	
"	" 13	1	3	$\frac{1}{5}$	"	2200	440	220,000	44,000	
"	" 13	1	3	$\frac{3}{5}$	"	4000	?	133,300	?	The majority, by far, were anthrax, but the separate estimate could not be made, as the plate was liquefying rapidly.
"	" 14	2	3	$\frac{1}{5}$	"	9600	320	960,000	32,000	
"	" 14	2	3	$\frac{3}{5}$	"	?	?	?	?	Uncountable, owing to the rapid liquefaction of plate.
"	" 16	4	3	$\frac{1}{5}$	"	2800	?	280,000	?	Chiefly, but not entirely, water forms.
"	" 16	4	3	$\frac{2}{5}$	"	2880	0	144,000	0	No anthrax visible.

Series D.

Table D (II).—Crude Thames Water infected forthwith with *Asporogene* Anthrax.

Date on which water was collected.	Date of making plate.	Number of days water stood at 20° C.	Number of days plate was incubated at 20° C.	Quantity of water used for plate.	Diluted or not.	Number of colonies on plates.		Calculated average number of bacteria per 1 c.c. of water.		Remarks.
						Total.	Anthrax.	Total.	Anthrax.	
May 12	May 12	0	7	c.c. $\frac{1}{5}$	$\frac{1}{20}$th dilution	Many	Many	?	?	9 water forms appeared (=900 per c.c.), and towards the fifth day very numerous anthrax.
,,	,, 12	0	7	$\frac{3}{5}$	,,	,,	,,	?	?	Here also 32 water forms soon appeared (=1066 per c.c.), but, though very numerous anthrax appeared towards fifth day, the plate was already liquefying badly.
,,	,, 13	1	5	$\frac{1}{5}$	,,	2,000	1000	200,000	100,000	
,,	,, 13	1	5	$\frac{3}{5}$	,,	,,	,,	,,	,,	Plate liquefied, but about the same numbers.
,,	,, 14	2	2	$\frac{1}{5}$	,,	13,680	0	1,368,000	0	Possibly anthrax not yet developed; a few appeared later, but could not estimate.
,,	,, 16	4	3	$\frac{1}{5}$	,,	2,800	0	280,000	0	The anthrax seemed to have entirely disappeared.
,,	,, 16	4	3	$\frac{2}{5}$	,,	2,080	0	104,000	0	,, ,, ,,
,,	,, 18	6	3	$\frac{1}{5}$	,,	580	0	58,000	0	,, ,, ,,
,,	,, 18	6	3	$\frac{2}{5}$	,,	1,345	0	67,250	0	,, ,, ,,

Series D.

Table D (III).—Thames Water filtered forthwith through Porcelain, and infected with *Virulent* Anthrax.

Date on which water was collected.	Date of making plate.	Number of days water stood at 20° C.	Number of days plate was incubated at 20° C.	Quantity of water used for plate.	Diluted or not.	Number of colonies on plate.		Calculated average number of bacteria per 1 c.c. of water.		Remarks.
						Total.	Anthrax.	Total.	Anthrax.	
				c.c.						
May 2	May 12	0	3	$\frac{1}{5}$	$\frac{1}{20}$th dilution	224	200	22,400	20,000	The few other forms were a yellow intruder.
,,	,, 12	0	3	$\frac{3}{5}$	,,	Too many to count	All anthrax	?	?	Not fewer than last.
,,	,, 13	1	5	$\frac{1}{5}$	,,	80	80	8,000	8,000	On the third day the colonies were so small that we could not count them: the (pure) cultures were, therefore, kept longer.
	,, 13	1	5	$\frac{3}{5}$	,,	360	360	12,000	12,000	
,,	,, 14	2	4	$\frac{1}{5}$	,,	200	200	20,000	20,000	
,,	,, 14	2	4	$\frac{3}{5}$	,,	360	360	36,000	36,000	
,,	,, 16	4	4	$\frac{1}{5}$	,,	220	220	22,000	22,000	
,,	,, 16	4	4	$\frac{2}{5}$	,,	..	..	..	..	Plate spoilt.
,,	,, 18	6	4	$\frac{1}{5}$	,,	200	200	20,000	20,000	

On comparing the Tables D (I) and D (II), we see that the water forms pass through the usual stages of rapid rise in numbers during the first two or three days, followed by a relapse to fewer and fewer; but it is suggestive that the enfeebled "asporogene" anthrax seemed to disappear more rapidly from the plates—Table D (II). This rapid disappearance did not occur where the water was sterilised by filtration, however, as Table D (III) shows, and I was for some time inclined to attribute this to the influence of the normal aquatic bacteria. It may still turn out to be so, but my experiments on p. 290 do not support the idea, and our confidence being shaken in the character of the "asporogene" anthrax used, I do not press the point, but hope to raise the question in another form at a later date.

The evidence goes to show, then, that *Bacillus anthracis*, while *only capable of living for a short time in the Thames water in the vegetative state, is able to persist very much longer in the form of spores.*

Bacteriological Examination of some Old Culture Flasks.

On October 6, it was decided to analyse the contents of five flasks, selected from a series which had been put aside for this purpose on the 5th March previous. The analysis of these five flasks is very interesting.

The flasks were labelled A, B, B′, C, and D, and had been treated in various ways, as described below.

A was a litre flask, which on March 5 had been charged with about 600 c.c. of fresh Thames water, inoculated with a comparatively large dose (10 c.c.) of the same water, in which a vigorous potato culture of anthrax had been shaken up. The culture used was known to contain spores, and a good deal of starch had also been carried over.

The flask stood during the whole of the period (spring and summer) under a glass bell-jar, on a table near a north window, and received no direct sunlight, but ordinary bright daylight every day.

It was occasionally opened (the cotton-wool plug being carefully and quickly removed and replaced each time), to remove samples for analysis.

The temperature rarely fell below 12° C., or rose beyond 15° C., and a slight growth of green microscopic algæ made its appearance during the summer.

On October 6 cultures were made (1) direct from the flask (2) from same after exposure to 56° C. for 24 hours.

The direct cultures on gelatine gave plenty of bacteria, &c., but if any anthrax was there it was overwhelmed by the alien forms, or would not grow.

The cultures, after exposure at 56° C., gave no results on gelatine, but animals inoculated with 2 c.c. died in five days of anthrax.

The results (see Table F) showed that spores had remained alive for seven months in the flask, and though enfeebled they were capable of germinating and killing.

Flask B, *March* 5 *to October* 6, 1892.—B was also a 1-litre flask, filled with about 500 c.c. Thames water, and inoculated with 8 c.c. of the anthrax infection.

Kept in the open laboratory. Cotton-wool plug. Under bell-jar. At east window. Partial insolation in mornings. Temperature varied from 12°to 20° C.

After being thus undisturbed till October 6—*i.e.*, seven months—the plates showed no anthrax, though numerous other (water) bacteria were present.

A guinea pig inoculated with 2 c.c. of the water did not die in fourteen days.

Cultures from plates of the water, after exposure at 56° for twenty-four hours, showed no anthrax.

This result shows either (1) insolation killed off the spores, or (2) the temperature was not high enough for spores to form. That the first suggestion is the right one will be shown in the sequel.

Flask B′, *March* 5 *to October* 6, 1892.—1-litre flask of Thames water exactly like B, and inoculated in the same way.

The only difference in treatment was that B′ was placed at 20° C. in the incubator, and remained two months at that temperature; then, still in incubator, it was left through the summer in the dark, at the same temperature as B.

Plates on October 6 (*i.e.*, seven months later) gave no anthrax, either from water direct or after twenty-four hours at 56° C. Any anthrax-like colonies turned out to be saprophytes.

Nevertheless a guinea pig inoculated with 2 c.c. of the water died in three days, and cultures of anthrax were made from the heart.

Suggests that spores developed well, and kept well, but that they were either too few or too feeble to be easily detected on gelatine.

Flask C, *March* 5 *to October* 6, 1892.—1-litre flask filled with Thames water and boiled for half an hour, and then inoculated with 2 c.c. (therefore water organisms introduced), exactly as before.

Kept at 20° C. for two months in dark incubator, then remained (in same) for five months at ordinary temperatures.

On October 6 plate cultures gave no anthrax, though other organisms were shown. After twenty-four hours at 56° C., plates also gave no results; but a guinea pig inoculated with 2 c.c. of the water died in five days, and the heart's blood gave cultures of anthrax.

Suggests that boiling the Thames water in no way hurts it as a medium for anthrax to sporify in.

Flask D, *March* 5 *to October* 6, 1892.—1-litre flask filled with crude Thames water, and not inoculated.

Kept in incubator at 20° C. for two months, then (same place) at ordinary temperature for five months more.

Plates gave no anthrax, either raw or after the water was at 56° C. for twenty-four hours, yet other organisms were found.

A guinea pig inoculated with 2 c.c. of the water lived.

The experiment suggests that there was no anthrax in the original Thames water, or it would have made itself evident during the sojourn in the incubator.

These results are summarised in the following table :—

Table F.—Examination of Flasks A, B, B[1], C, and D of March 5 to October 6.

Flask.	Contents of flask.	Date on which water was collected.	Date of making plates.	Number of days the water had stood.	Exposed to light or not.	Temperature at which water stood.	Time plate was incubated.	Quantity of water used for plates.	Diluted or not.	Number of colonies on plates. Total.	Number of colonies on plates. Anthrax.	Remarks.
						° C.	days.	c.c.				
A	raw water infected	Mar. 5	Oct. 6	216	ordinary daylight	10—15	5	$\frac{1}{30}$	$\frac{1}{10}$th dilution	1	0	Corresponds to 300 bacteria per 1 c.c.
,,	,,	,,	,,	,,	,,	,,	8	,,	,,	7	0	One of the colonies, suspiciously like anthrax when young, was isolated and proved not anthrax.
,,	,,	,,	,,	,,	,,	,,	5	$\frac{4}{30}$	,,	3	0	Corresponds to 225 bacteria per 1 c.c.
,,	,,	,,	,,	,,	,,	,,	8	,,	,,	15	0	
B	,,	,,	,,	,,	east window	12—20	5	$\frac{1}{30}$	,,	35	0	Three colonies very like typhoid in appearance, but isolated and proved different.
,,	,,	,,	,,	,,	,,	,,	8	,,	,,	22	0	
,,	,,	,,	,,	,,	,,	,,	5	$\frac{4}{30}$	,,	90	0	
,,	,,	,,	,,	,,	,,	,,	8	,,	,,	100	0	One anthrax-like colony proved to be not anthrax.
B[1]	,,	,,	,,	,,	in dark	20*	5	$\frac{1}{30}$	,,	40	0	Nine liquefying.
,,	,,	,,	,,	,,	,,	,,	8	,,	,,	26	0	
,,	,,	,,	,,	,,	,,	,,	5	$\frac{4}{30}$	,,	200	0	
,,	,,	,,	,,	,,	,,	,,	8	,,	,,	115	0	Several badly liquefying.

* In this case the flask was at ordinary summer temperature, 14—20° C., after August 1, but in dark incubator.

Table F—*continued.*

Flask.	Contents of flask.	Date on which water was collected.	Date of making plates.	Number of days the water had stood.	Exposed to light or not.	Temperature at which water stood.	Time plate was incubated.	Quantity of water used for plates.	Diluted or not.	Number of colonies on plates.		Remarks.
										Total.	Anthrax.	
						° C.	days.	c.c.				
C	boiled water infected	Mar. 5	Oct. 6.	216	ordinary daylight	10—15	5	$\frac{1}{30}$	$\frac{1}{10}$th dilution	6	0	It is interesting to note how much fewer the water colonies were in the flask of *boiled* water.
,,	,,	,,	,,	,,	,,	,,	8	,,	,,	9	0	
,,	,,	,,	,,	,,	,,	,,	5	$\frac{4}{30}$	,,	10	0	
,,	,,	,,	,,	,,	,,	,,	8	,,	,,	24	0	
D	raw water not infected	,,	,,	,,	,,	,,	5	$\frac{1}{30}$	,,	4	0	
,,	,,	,,	,,	,,	,,	,,	8	,,	,,	10	0	
,,	,,	,,	,,	,,	,,	,,	5	$\frac{4}{30}$	,,	40	0	
,,	,,	,,	,,	,,	,,	,,	8	,,	,,	48	0	

On October 26, 1892, we examined the contents of a flask which had stood since March 5, 1892, in a north window of the laboratory, *i.e.*, it had remained nearly eight months undisturbed, at ordinary temperatures and in diffused daylight.

This flask was an iuteresting one in many respects. When first placed in position, on March 5, it had received a charge of about 300 c.c. of fresh Thames water, infected with a very strong charge of virulent anthrax taken from a potato culture, but we discarded it at the time because (1) the culture was found to contain so many spores, and (2) so much of the starch of the potato had been transmitted with the charge that we judged it better to renew the experiments we were engaged in.

During the summer the water in this flask became quite green with microscopic algæ, evidently developed from the Thames water, and it seemed worth while on October 26 to test the water for anthrax, to see if the presence and activity of the algæ had eliminated that organism.

The positive results of the analysis are shown below in Table G. On several of the plates no anthrax could be found at all; but in the two cases recorded in the table there was no doubt whatever.

Table G.—Bacteriological Analysis of Stock Flask of Thames Water infected with Anthrax and green with Algæ after standing at ordinary temperatures from March 5 to October 26 in diffused light.

Date of making plate.	Quantity used for plate.	Diluted or not.	Number of colonies on plate.		Number of bacteria per 1 c.c. of original.		Remarks.
			Total.	Anthrax.	Total.	Anthrax.	
26.10.92	c.c. $\frac{1}{30}$	$\frac{1}{11}$th dilution	200 to 300	1	66,000 to 99,000	330	Only one *undoubted* anthrax colony could be found. Total absence of liquefying bacteria noteworthy.
	$\frac{6}{30}$	”	800 to 12,000	1	44,000 to 66,000	55	Again, only one anthrax colony could be discovered with certainty.

Two other facts are worth note in these analyses. (1) The large number of water organisms which had persisted through the seven to eight months, and (2) the total absence of rapidly liquefying forms.

It was already clearly proved then that the presence of the green algæ and the diffuse daylight had not exterminated the anthrax, although the numbers were extremely diminished, for on starting the experiment our flask contained something like 1,000,000 per 1 c.c., and more.

To place the matter still further beyond doubt, however, we placed 25 c.c. of the water at 56° C. for twenty-four hours, and obtained 1 anthrax colony in a 3-drop plate, and several plates with no trace of anthrax; then we got a 3-drop plate, among several with negative results, showing 5 anthrax colonies, and finally a 12-drop plate with 38 anthrax colonies.

It was observed that in all these cultures the anthrax colonies came on very slowly, and had there been any liquefying bacteria present, it is practically certain that we should have missed the anthrax altogether.

The conclusion is inevitable that although the anthrax had not been eliminated from this flask, it had been enormously diminished in quantity, and enfeebled as regards the powers of germination of the spores.

This conclusion was made a certainty by the following test:—On November 7 a guinea-pig was inoculated intra-peritoneally with $2\frac{1}{2}$ c.c.* of the water in the flask; at first we thought it had escaped, but it died on November 17. Cultures from the heart's blood proved that it had died of anthrax; but it took ten days for the feeble and few spores to do the work.

The chief interest attaching to this series of experiments, however, is the proof that *insolation rapidly rids the water of the spores of anthrax.* I shall show later on that this is not only a very definite action, but one capable of being more directly and easily demonstrated than has hitherto been suspected.†

Experimental Observations on the Bacterial Flora of the Thames.

As already pointed out (p. 244), I have devoted considerable attention to the normal aquatic bacteria of the river water, isolating each form for further culture as it turned up in the course of the

* This large dose was given because we found so few spores, and these apparently very much enfeebled.

† See our First Report ('Roy. Soc. Proc.,' vol. 51, 1892), pp. 199 and 237, for the literature dealing with the action of light on bacteria. See also pp. 303 and 310 of this Report.

investigation. Indeed, some of the first questions I set myself were the following:—(1.) What Schizomycetes are ordinarily found in the water? (2.) Are any of them pathogenic? (3.) Does *Bacillus anthracis* occur in the Thames? And (4) how do the aquatic forms behave in cultures?

Our preliminary examinations showed that several forms of Schizomycetes can be distinguished as common in the water, while here and there a yeast and a mould have been met with. I shall defer the consideration of all the other forms, with the exception of the one treated below.

Of these several forms we have been strongly impressed with the characters of some, while others have shown such slight individuality that it is difficult to be sure of their autonomy.

Of the well marked forms, one very common one is particularly characterised by its rapid growth and liquefaction of the gelatine, with a greenish hue and slightly putrid odour. This form is very like one of the forms known as *Bacterium termo*, and separated by Macé as *Bacillus termo*.* For some time we thought this was the species referred to, but prolonged and careful isolations and cultures have shown quite clearly that it is the form described by Flügge under the very descriptive name of *Bacillus fluorescens liquefaciens*.†

It occurs on the gelatine plates at all ordinary temperatures up to 20° C., as minute, greyish-white points, which rapidly enlarge to circles, and soon begin to liquefy, so that the colony lies at the base of a perfectly circular concave depression as a granular flocculent mass with a tinge of green, and with irregular radiations or networks into the liquefied circular area.

In from twenty-four to forty-eight hours the area of liquefaction extends very rapidly—in forty-eight hours at 12—15° C. the colonies were each as big as a shilling—and soon floods the plate with a slightly malodorous slimy fluid, of a pale emerald-green hue, with a very distinct fluorescent shimmer.

Such colonies consist of very short fine rods, often with a slight constriction, or in couples, and then difficult to distinguish from chains of cocci, from 2 to 3·5 μ long by 0·5 to 0·8 μ broad (measured after staining in Spiller's purple and mounting in Canada balsam); in no case have we found filaments or spores in the gelatine cultures.

If transferred to gelatine tubes, the same depression, rapid liquefaction, and green fluorescence are observed at 12°, 15°, and 20° C.; and if the culture is made as a "stab," a very characteristic series of events follow. The liquefaction of the gelatine proceeds so as to form a funnel, very like a "thistle-head" in shape, and the flocculent greyish-white colonies fall slowly to the bottom of the rapidly

* Macé, 'Traité pratique de Bactériologie,' pp. 585—587.

† Flugge, 'Die Mikroorganismen.'

widening "stem," which is filled with the liquid. The peculiar green fluorescence is very marked in these cases, masking somewhat the true colour of the colony itself.

On agar the colonies rapidly spread at 20—25° C. (more slowly at 12—15° C.), as a thin, wet-looking, or almost waxy, greenish-white layer, becoming thicker eventually, and very smooth and glassy, and tinging the subjacent agar with the characteristic hue. Here and there longer rods can be found in agar cultures, but no trace of spore formation could be discovered either on this or any other medium.

On potato the colonies are brownish-yellow, becoming deeper with age, and often with a raised, rough, granular surface, moist or oily in appearance. On all the solid media the fact that this bacillus is strictly aërobic comes out very strongly. Its growth is at once inhibited if a sterilised glass cover slip is placed over the young colony: the liquid rapidly fills up all the interspaces, and no air can enter, and growth stops at once. Moreover, if a "stab" culture is carefully made and covered with gelatine, the same inhibition is noticed.

The marked and rapid disappearance of this form from water which is kept standing in a closed vessel is almost certainly to be attributed to the same cause.

We have made numerous attempts to cultivate this form in hanging drops of gelatine, and with success, but there are no special points to notice: the rods divide very rapidly, and never grow out into long filaments or form spores in the moist chambers. It is impossible to cultivate it under a cover slip in compressed gelatine.

Milk is rendered slightly acid by the bacillus, and coagulation and peptonisation follow.

The slimy, green, fluorescent liquid presents several interesting features. Slight quantities of acid—hydrochloric or acetic—cause the green colour to disappear; but neutralisation with ammonia at once restores it, and an excess of the alkali deepens the hue. The colour is not destroyed by boiling, though, if prolonged, the green hue becomes distinctly paler. This agrees exactly with Macé's account of it, and is no doubt strong confirmatory evidence as to the correctness of our identification of the Schizomycete.

The annexed table summarises the character of this Schizomycete.

Characteristics of *Bacillus fluorescens liquefaciens* (Flügge).

Habitat.....	Thames water at all seasons.
Morphological characters	Very small short rodlets, average size $1—1\cdot5\,\mu \times 0\cdot5\,\mu$, sometimes in pairs or short chains, and often slightly constricted.
Spores......	None found in any medium.
Colonies on gelatine plates	Small, circular, rapidly enlarging to the size of a shilling and more, and then forming liquefied depressions with perfectly circular clear edges, and flocculent greenish-white granular masses of the organism floating in the centre, often with networks radiating from denser centre.
In gelatine tubes	*Streak cultures* rapidly liquefying at all ordinary temperatures, and the liquefied slimy gelatine with a yellowish-emeral hue, fluorescing greenish-blue. Slight putrefactive odour. *Stab cultures* very characteristic. The liquefaction begins above, forming a concave funnel-like depression, and extends down the puncture; in two or three days a "thistle-head" funnel of liquefied gelatine, very green and fluorescent, especially above. The greyish flocculent colonies gradually settle down the stem of the "funnel," widening the area of liquefaction above.
On agar	At all ordinary temperatures to 20—25° C., spreading as a greenish-white wet layer, at first thin. The fluorescent green tinge penetrates a couple of millimetres or more into the agar, which remains solid.
On potato....	In five days at 20—25° C. forms a yellowish-brown, and shining, often granular layer, deepening in colour subsequently.
In bouillon..	Turbidity, green fluorescence, and deposit.
In milk.....	Precipitates the casein, and then completely peptonises it. Acid reaction. Liquid clear in 10—14 days at ordinary temperatures.
Temperature	Grows well at all temperatures from 10° C. to 25° C. Cardinal points not determined.
Rapidity....	Very rapid development and liquefaction. It is the earliest form to appear on gelatine plates.
Air requirements	Markedly aërobian. Will not grow under glass plates, or submerged in solid media.
Light.......	Grows well in the dark.
Pigment	Soluble in the medium. Becomes paler on boiling, but is not destroyed. Disappears at once if acidified with hydrochloric or acetic acid, but reappears on neutralising with ammonia; an excess of alkali deepens the green fluorescent hue.
Pathogenic or not	Not. It is a chromogenic saprophyte, and grows well in water containing mere traces of soluble organic materials.

If we now attempt to discover this form among the species recorded in Eisenberg, Macé, and Roux, there appear to be only the following to choose from as the known liquefying forms which produce the characteristic green fluorescence in gelatine, or which possess any green pigment at all:*—*Bacillus fluorescens liquefaciens* (Flügge), *B. fluorescens liquefaciens minutissimus* (Unna), *B. fluorescens nivalis* (Schmolck), *B. viscosus* (Frankland), and *B. termo* (Duj. and Macé). We will also notice *B. aërophilus* (Libor.) and *B. chlorinus* (Engelm.) as being accompanied by a green colour in the cultures. We may at once eliminate *B. chlorinus*, however, because, according to Engelmann's description (he called it *Bacterium chlorinum*), the green colouring matter is in the cells, and these are much too large for our form. The same applies to Van Tieghem's *Bacillus viridis* and *B. virens*, and we are not concerned here with the discussion as to the chlorophyll nature of the colour or the claims of these forms to be regarded as Schizomycetes at all.

B. aërophilus may also be readily eliminated, for, apart from the large size of its filaments, it grows very slowly, and does not colour the gelatine; its green colour is confined to the colonies.

B. fluorescens liquefaciens (Flügge) presents startling points of similarity with our form.

It agrees in habitat, water and air, &c., as well as in the mean size and union of the rodlets, and is motile.

The colonies on gelatine plates are, like our form, round and depressed in funnel form, and the very regular clear zone of liquefaction is characteristic, as also the mode of liquefaction and fluorescence.

On agar, too, according to Macé, the growths are quite similar, while the very marked aërobian character and rapid growth are also alike.

In short, we find no important differences between the descriptions, and therefore regard this form as identical with Flügge's *B. fluorescens liquefaciens*.

B. fluorescens liquefaciens minutissimus (Unna), whether a good species or not, presents sufficient differences to separate it from ours. Apart from its habitat, the feeble fluorescence and capacity for growing anaërobically would seem to separate it.

B. fluorescens nivalis (Schmolck) is a form found in the glacier waters of Norway, and reminds one forcibly of *B. fluorescens liquefaciens*. The information to hand is too meagre to enable us to decide.

B. viscosus (Frankland) is possibly eliminated by the characters of the colonies, if the author's description of the radiating hair-like marginal growths is characteristic. Nor does the size quite agree, though the discrepancies could hardly be insisted upon. In other

* See our First Report, Appendix B, for literature concerning these forms.

respects, however (*e.g.*, as regards habitat and mode of liquefaction, &c.), there are suggestive resemblances, and Macé regards it as identical with *B. fluorescens liquefaciens.*

Bacillus termo is the name given by Macé to a form which he separated from the mixture which previously passed under the name of *Bacterium termo* (Dujardin), and it presents several impressive similarities to our form.

It is very common in water, and has the size and shape and modes of union and movement observed. In fact almost the only serious discrepancy we find as regards the habit of the two forms is that ours has not the peculiar wavy or "amœboid" contour of Macé's form.

As will readily be understood, the critical examination of these water bacteria is a matter of time, and requires much care; nevertheless, I regard it as of great importance to the object we have in view, and propose now to give an example of one of the lines of inquiry which ought to be pursued as closely as possible, and which can only be properly pursued when the separate water organisms have been thoroughly studied.

Experiments on the Behaviour of Anthrax sown simultaneously with B. fluorescens liquefaciens *in Water.*

For many reasons, and especially because this is a common water form, perhaps the commonest Schizomycete in the Thames, it seemed worth while to try the effect of sowing *Bacillus anthracis* and this *B. fluorescens liquefaciens* together in the same water. The following experiments were accordingly carried out:—

A litre flask was filled about three-quarters full (700 c.c.) of sterilised distilled water and plugged with cotton wool, and the whole carefully sterilised and allowed to cool, and a very dense sowing of a mixture of active anthrax and of *B. fluorescens* put into the water. The sowing was accomplished as follows:—10 platinum loops full of a vigorous two-day agar culture of anthrax were rubbed into a sterile test-tube with 1 c.c. of sterilised distilled water; then 10 loops of a *B. fluorescens* culture as equal as possible were treated in the same way in another tube, and the contents of the two tubes mixed and shaken thoroughly. 1 c.c. of this mixture was finally put into the 700 c.c. of the sterilised distilled water and thoroughly mixed by shaking. The flask was then placed in the dark incubator at 20° C. Every effort was made to introduce as little of the culture media (agar) as possible, and to sow approximately equal quantities of each Schizomycete, though of course it was impossible to attain these objects completely. Then plate cultures were made day after day—starting with one made directly on sowing the bacteria—to see what, if any, effect the struggle for existence would present.

The results are shown in the annexed table:—

Table z.—*B. anthracis* and *B. fluorescens liquefaciens* together in Distilled Water, at 20° C., in the dark.

Number of days flask stood.	Date of making plate.	Number of days plate was incubated at 20° C.	Quantity of water used for plate.	Diluted or not.	Number of colonies found on plate.		Calculated total number of bacteria per 1 c.c. original.		Remarks.
					Anthrax.	*B. fluorescens.*	Anthrax.	*B. fluorescens.*	
			c.c.	Diluted					
0	Nov. 16	3	$\frac{1}{25}$	49 : 1	3	2	3750	2500	
1	,, 17	3	$\frac{1}{25}$	,,	3	4	3750	5000	
2	,, 18	3	$\frac{1}{25}$	,,	2	1	2500	1250	
3	,, 19	3	$\frac{1}{25}$	,,	1	0*	1250	0	*1 *Mould.*
5	,, 21	4	$\frac{1}{25}$	24 : 1	2	2	1250	1250	Badly liquefied.
6	,, 22	3	$\frac{1}{5}$	,,	28†	13	3500	1625	† A little doubt as to *all* being *anthrax?*
6	,, 22	3	$\frac{1}{5}$	,,	15	18	1875	2250	
9	,, 25	3	$\frac{1}{25}$	29 : 1	1	0‡	750	0	‡1 *Sarcina* (intruded ?).
9	,, 25	3	$\frac{1}{25}$	,,	0	0	0	0	
10	,, 26	3	$\frac{1}{25}$	,,	0§	0	0	0	§1 *mould* (intruded ?).
13	,, 29	3	$\frac{1}{25}$	Not diluted	17	0	425	0	
15	Dec. 1	8	$\frac{1}{25}$	,,	2	0‖	50	0	‖1 *mould* and 2 colonies of an intruded form.
15	,, 1	8	$\frac{1}{25}$	,,	0	0	0	0	
18	,, 4	5	$\frac{1}{4}$	,,	2	0	8	0	These four plates were made by pouring a large tube of gelatine infected with 1 c.c. of the water, into four plates. Average ∴ = 34 per 1 c.c.
18	,, 4	5	$\frac{1}{4}$	,,	24	0	96	0	
18	,, 4	5	$\frac{1}{4}$	,,	0	0	0	0	
18	,, 4	5	$\frac{1}{4}$	,,	8	0	32	0	
28	,, 14	5	$\frac{1}{5}$	,,	7	0	35	0	Contents of a 10-drop tube on two plates. Average = 30 per c.c.
28	,, 14	5	$\frac{1}{5}$	,,	5	0	25	0	
37	,, 23	5	$\frac{2}{5}$	,,	2	0	5	0	
42	,, 28	..	$\frac{2}{5}$	,,	33	0	82	0	

On carefully going over this table, which illustrates very clearly the results of the struggle for existence between the two organisms, it is evident (1) that both the anthrax and the *Bacillus fluorescens liquefaciens* fall off in numbers day after day—for it cannot be urged that the doubtful high number found on the sixth day invalidates the general results—and (2) that the anthrax persists simply because it passes into the spore condition.

To make this last point quite certain, we heated a few c.c. of the water to 54° C. for twenty-four hours, and then made new plates, with the expected result: there were anthrax spores present.

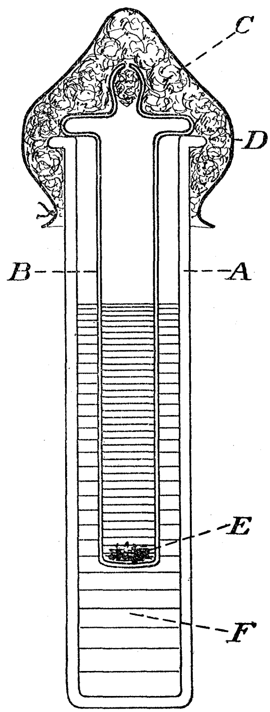
Possibly the third inference to be drawn from this experiment is the most interesting of all, viz., that *anthrax can hold its own in distilled water at 20° C., in the dark, not only in spite of the presence of* B. fluorescens, *but (owing to its power of forming spores) it may even tire out and exterminate the latter, because it cannot form spores in the water.*

These results show with startling clearness how cautious we must be in forming any opinions about the mutual relations between any two bacteria, or, indeed, between any two organisms whatever. The whole subject is a matter of biological experiment, and cannot be predicted on any other grounds than those of actual experiment. It is very commonly supposed that a saprophyte like *B. fluorescens liquefaciens* must be able to drive out a parasite like *B. anthracis* if the latter invades the territory of the former; the above experiment shows that such is by no means the case under the conditions afforded, and we ought to be very cautious indeed in surmising what will occur under other conditions of the same general kind.

In view of the facts above recorded, it seemed advisable to test the question in another way, and we did this as follows:—

A sterilised glass cylinder, *A*, receives a porcelain filter, *B*, also sterilised, and held in position and protected from dust, &c., by a covering and plug of sterilised cotton wool, *C*, and a paper cap. After final sterilisation, the cylinders received about 300 c.c. of Thames water, sterilised by filtration through porcelain. *Bacillus fluorescens liquefaciens* was sown in the water *inside* the filter *B*, and *anthrax* in the water *outside* the porcelain. Since experiments showed that the green fluorescing liquid of the former passes readily through such a filter, and that the excretions made by anthrax do the same, it was hoped that this apparatus would help to answer the question, Do either of these bacilli poison the water for the other?

The results, after twenty-four days' incubation at 20° C. in the dark, proved that both the organisms were still alive, the anthrax being chiefly or entirely in the form of spores. On heating the whole apparatus up to 56° C. for twelve hours, the plates showed total absence of *B. liquefaciens fluorescens*—proving that it developed



A, glass cylinder; *B*, Chamberland filter; *C*, sterilised cotton wool; *D*, sterilised paper cap tied over the cotton wool; *E*, *Bacillus fluorescens liquefaciens*; *F*, *Bacillus anthracis*.

no spores—while *anthrax was present after this treatment to the extent of many thousands per cubic centimetre of the liquid.*

Clearly, therefore, the anthrax is not poisoned off by the secretions of *B. fluorescens liquefaciens.*

These results suggested the following simple experiment:—

Four sets of test-tubes, properly prepared, were arranged as follows:—

One set (A) received a charge of the green fluorescing liquid obtained by allowing *Bacillus fluorescens* to thoroughly liquefy gelatine, the charge being simply filtered at ordinary temperatures through sterilised filter paper; this merely holds back the large flocculent masses of the Schizomycete, and allows numerous isolated ones to pass.

Set B received an exactly similar charge, but was then put into a beaker of water kept boiling for fifteen minutes.

A third set (C) was charged with equal quantities of ordinary nutrient gelatine and of the above green liquid.

The fourth set (D) was prepared exactly as C, but kept in boiling water for fifteen minutes.

When all were ready, and the heated tubes had cooled to 25—26° C. just sufficient to solidify the gelatine, a large loopful of anthrax spores was placed in each tube, and all four sets put into the dark incubator at 20° C.

The results were less satisfactory, as regards sharpness, than we hoped, but it was clear that the spores of anthrax were still there, and alive in all the tubes, after three weeks.

Finally, the following careful set of experiments were made :—

A quantity of Thames water, collected on the morning of December 10, was at once filtered through a Chamberland porcelain tube at low pressure, the whole apparatus having been very carefully sterilised. Control experiments showed that this sample of water was almost entirely devoid of water organisms.

Two similar flasks were prepared, and into these 1 litre of the above water was distributed after the water had been infected as follows :—

To the 1 litre of filtered water 1 c.c. of a liquefied gelatine culture of anthrax, consisting almost entirely of rodlets and filaments, but partly of spores, and 1 c.c. of a similar liquid culture of *B. fluorescens liquefaciens* were added, and thoroughly shaken up.

Each charged flask was then placed in the dark, one at 12° C., the other at 20° C., and plates made daily, as shown in Table *y*, which summarises the results.

Table *y*.—Behaviour of *Anthrax* and *Bacillus fluorescens liquefaciens* growing together in Filtered Thames Water at 20° C. and 12° C. respectively.

Number of days flask stood.	Temperature at which flask stood.	Date of making plate.	Number of days plate was incubated.	Quantity of water used for plate.	Diluted or not.	Number of colonies found on plate. Anthrax.	Number of colonies found on plate. *B. fluorescens.*	Calculated number of bacteria in 1 c.c. original. Anthrax.	Calculated number of bacteria in 1 c.c. original. *B. fluorescens.*	Remarks.
	° C.			c.c.						
0	..	Dec. 10	2	$\frac{1}{50}$	Not	100	100	5,000	5,000	Average = 3958 *anthrax* and 4916 *B. fluorescens* per 1 c.c.
0	..	”	2	$\frac{1}{50}$	”	50	90	2,500	4,500	
0	..	”	2	$\frac{1}{25}$	”	100	110	2,500	2,750	
0	..	”	2	$\frac{1}{25}$	”	35	100	1,875	2,500	
1	20	Dec. 11	2	$\frac{1}{25}$	”	4000	1,000	100,000	25,000	The plates with $\frac{2}{25}$ c.c. and $\frac{3}{25}$ c.c. were absolutely beyond counting, but enough was seen to assure me that 100,000 *anthrax* and 25,000 *B. fluorescens* is not too high an average.
1	”	”	2	$\frac{2}{25}$	”	?	?	?	?	
1	”	”	2	$\frac{3}{25}$	”	?	?	?	?	
1	12	”	2	$\frac{1}{25}$	”	170	64	4,250	1,600	The 3rd plate was too much liquefied, but the numbers were not less than 2nd average = 2035 *anthrax* and 585 *B. fluorescens.*
1	”	”	2	$\frac{3}{25}$	”	600	300	5,000	2,500	
1	”	”	2	$\frac{3}{25}$	”	?	?	?	?	
2	20	Dec. 12	2	$\frac{1}{25}$	”	800	3	20,000	75	Again must take average from one plate: the other too far liquefied *by the anthrax colonies.*
2	”	”	2	$\frac{2}{25}$	”	?	?	?	?	
3	20	Dec. 13	2	..	..	..	..	..	..	Plate lost.

Table *y*—*continued.*

Number of days flask stood.	Temperature at which flask stood.	Date of making plate.	Number of days plate was incubated.	Quantity of water used for plate.	Diluted or not.	Number of colonies found on plate.		Calculated number of bacteria in 1 c.c. original.		Remarks.
						Anthrax.	*B. fluorescens.*	Anthrax.	*B. fluorescens.*	
	° C.			c.c.						
3	12	Dec. 13	2	$\frac{1}{25}$	Not	?	?	?	?	Countless thousands, and badly liquefied, but the type neither that of *anthrax* nor *B. fluorescens.*
4	20	Dec. 14	2	$\frac{2}{25}$	Diluted 5 : 1	?	Total = 1,500	?	Total = 112,500	Most = *B. fluorescens,* but could not estimate proportions.
4	20	,,	2	$\frac{2}{25}$	,,	?	Total = 1,200	?	Total = 90,000	*Anthrax* preponderated, but could not get proportions.
4	12	,,	2	$\frac{2}{25}$	,,	?	Total = 12,000	?	Total = 900,000	By far the majority = *B. fluorescens* in both sets; *anthrax* present.
4	12	,,	2	$\frac{2}{25}$	,,	?	Total = 10,000	?	Total = 750,000	
5	20	Dec. 15	2	$\frac{2}{25}$	,,	?	1,000	?	Total = 75,000	*Anthrax* present, but much dominated.
5	12	,,	2	$\frac{2}{25}$	,,	?	?	?	?	Utterly liquefied. *Anthrax* present, however.
7	20	Dec. 17	2	$\frac{1}{25}$	No dilution	2700	300	67,500	7,500	? *B. fluorescens* under estimated.
7	12	,,	2	$\frac{1}{25}$	,,	?	Total = 5,000	?	Total = 125,000	Majority by far = *B. fluorescens,* but not typical.
8	20	Dec. 18	2	$\frac{1}{25}$	,,	400	200	10,000	5,000	Quite typical, and number too low rather than too high.
8	12	,,	2	$\frac{1}{25}$	,,	?	?	?	?	Too far liquefied to count—many thousands—few *anthrax.*

10	20	Dec. 20	2	$\frac{1}{25}$	,,	?	Total = 542	?	Total = 13,550	
10	12	,,	2	$\frac{1}{25}$	,,	?	Total = 3,000	?	Total = 75,000	Almost all *B. fluorescens*, but *some anthrax* recognised.
13	20	Dec. 23	3	$\frac{1}{25}$	,,	?	Total = 900	?	Total = 22,500	Foreign forms intruded.
13	12	,,	3	$\frac{1}{25}$	,,	?	Total = 1,200	?	Total = 30,000	Some *anthrax* present.
18	20	Dec. 28	3	$\frac{1}{25}$	,,	?	Total = 1,100	?	Total = 27,500	Several foreign forms. Anthrax still there.
18	12	,,	3	$\frac{1}{25}$	,,	?	Total = 550	?	Total = 13,750	

The table shows conclusively that the *anthrax was not exterminated, even after eighteen days at either temperature.*

On January 3, the flask kept at 20° C. was heated to 56° C. for twelve hours, and plates made. These plates, after three days' incubation at 20° C., showed that anthrax was still present to the extent of from 110 to 230 per 1 c.c. of the water in the flask.

If the numbers in Tables x, p. 254, and y, p. 295, are put in the form of curves, the ordinates representing the numbers of bacteria, we find in all cases that there is a large and rapid rise to a maximum during the first four days; the climax may be reached during the second day, or the third or fourth, but it is always relatively high, and usually soon reached. Then follows an equally rapid fall during the next twenty-four to forty-eight hours, succeeded by a slower one.

It is interesting to note that the mixed *Bacillus anthracis* and *B. fluorescens liquefaciens* behave very similarly *as a whole* at the lower temperature, 12° C., which is not favourable to the anthrax; but at the *higher* temperature of 20° C. it looks as if disturbances of various kinds occur which lead to a very different and irregular curve.

It seems almost certain that temperature is not the only factor at work here, for, although its effects are very distinct, as shown by comparing Tables x, y, and z, there must be other circumstances concurring to account for the very different heights of the curves, and times taken to reach this maximum.

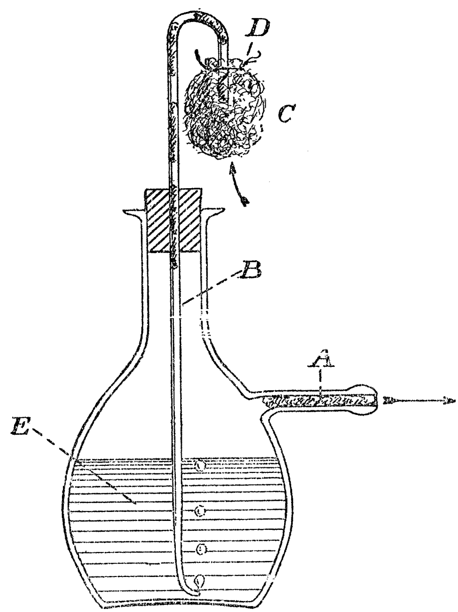
The extreme rapidity of the main ascent, when once it begins, suggests that a moment arrives when the ferment actions which must be supposed to render the food materials available are at their best; if this is so, the sudden fall may well be due to two causes—(1) the exhaustion of the available oxygen, and (2) that of the food materials themselves.

The actual height of the maximum— *i.e.*, the numbers of bacteria then sustained by the medium—may well be supposed to depend on a number of factors, *e.g.*, the quantity of food materials, especially organic, present in the water, the number of bacteria in the water at the outset, and, of course, the nature of both, being amongst the most important of these.

Obviously the whole subject is extremely complex, but we are inclined to think that valuable information could be got by extensive comparative examinations at constant temperatures of waters of known chemical composition infected with definite quantities of two known organisms, the behaviour of which should be studied at intervals of twelve hours if possible.

Experiments with Oxygenated (Aërated) Thames Water.

In order to test the action of combined movement and aëration, we employed the following means:—A flask of $1\frac{1}{2}$ litre capacity, and with a side arm tubulure, was half filled with the water to be experimented on, and its neck fitted with a caoutchouc stopper through which passed a long, narrow glass tube; the lower end of the tube was drawn to a point and passed to the bottom of the flask; the upper end was curved back on itself and carefully packed with successive plugs of sterilised cotton wool, and the end covered with a large tuft of the same. Preliminary trials convinced us that if this apparatus is properly sterilised, and carefully used, air may be drawn through the cotton-wool filter for two or three weeks without contamination of the contents. The accompanying figure represents the apparatus.



A, lateral tubulure plugged with cotton wool and attached to pump; *B*, curved tube plugged at intervals with cotton wool to filter air which passes into liquid below; *C*, large wad of cotton wool, tied on to air tube at *D*; *E*, water containing bacteria to be experimented with.

In the following series of experiments, one flask was aërated in the manner described for six days, and an exactly similar flask kept standing quietly by its side under exactly similar conditions otherwise. Both flasks contained Thames water, from the same collection,

and placed in action within a few hours of its removal from the river. The temperature of the room was 15—16° C., and remarkably constant day and night. The flasks were exposed to diffused daylight, and occasionally to the light of a Swan lamp.

After the six days the two flasks showed the following results on bacteriological analysis by means of gelatine plate cultures, using 1, 3, and 6 drops per plate, and diluting with 10 vols. sterile water to 1 vol. from flask. The numbers given are the calculated averages, and it is possible that more extended observations would, perhaps, alter them slightly.

State of water.	Number of drops in culture.	Total bacteria per 1 c.c. original.	Proportion of liquefyiug bacteria.	Remarks.
Aërated......	1	35,640	26 : 64	
Non-aërated ..	1	17,695	24 : 23	
Aërated......	3	33,000	26 : 64	
Non-aërated ..	3	15,840	1 : 3·5	
Aërated......	6	uncountable	..	Liquefied too rapidly for estimation.
Non-aërated ..	6	,,	..	

It must be remembered that in such experiments as these, the rapid development of the liquefying organisms is always the chief trouble, since we cannot allow the plates to incubate long enough to bring forward all the liquefying forms, on the one hand, while the liquefaction overpowers the young non-liquefying colonies on the other: consequently we lay no stress on the last column.

As regards the direct effect of the aëration, we believe the third column does express it more or less accurately, though of course the reply is always possible that we have no absolute guarantee that no aërial forms were filtered into the flask.

We propose to extend this inquiry at a later period, and merely put forward our results so far as tentative, and by no means devoid of interest and suggestiveness.

We next resolved to extend the comparison between aërated and non-aërated cultures to flasks of Thames water treated *exactly* as in the foregoing series except that we first infected both flasks with anthrax.

We employed a gelatine culture of the anthrax, selecting a tube of rapid growth, and in which the gelatine was completely liquefied, and used a relatively very large quantity (5 c.c. to the litre of water) and of course a correspondingly large quantity of gelatine food-material. This was done purposely in this first experiment, to en-

sure a distinct advantage to the anthrax in its struggle with the competing water forms.

The results are again expressed in calculated averages, in the following table. As before, the aëration was conducted for six days at 15—16° C., a temperature at which the spores can germinate.

State of water.	Number of drops in culture.	Total bacteria per 1 c.c. original.	Estimated proportion of anthrax to other bacteria.	Remarks.
Aërated......	1	Many thousands	1 : 10	
Non-aërated ..	1	Countless „	1 : 10	As far as could judge the proportions were similar.
Aërated......	3	..	1 : 20	
Non-aërated ..	3	..	..	Impossible to estimate.

Here it must be admitted that we failed in our attempt to ascertain any effects of the aëration on the anthrax as compared with the water organisms.

We, meanwhile, altered the course of the inquiry as follows:—Each flask, aërated and non-aërated, was placed, at the end of the six days, as soon as the samples had been removed for plate cultures, at 60° C. for twenty-four hours, and plates then made to ascertain, if possible, what had happened to the anthrax—*i.e.*, to see if spores had been formed, and to what extent in the two cases.

The following table summarises the results:—

State of water.	Number of drops used.	Calculated number of anthrax per 1 c.c. original.	Remarks.
Aërated...........	1	120,000	
Non-aërated	1	99,000	
Aërated...........	6	100,000	
Non-aërated	6	125,000	

So far as the quantitative results go, we regard the experiments here summarised as failures, because no stress whatever may be laid on the actual numbers until we have made a larger series on these comparative lines.

We do think, however, that the results are valuable in another

sense; for they show quite clearly that (1) the oxygenation and movement of the water for six days in diffused light does not sensibly reduce the anthrax if it passes into the spore stage, and (2) does not by any means eliminate the element of a struggle with normal water forms.

We regard the subject as well worth more exact study, and especially along lines to determine more accurately the relative direct effects on the various organisms in the water.

The following series was designed as a continuation of this inquiry.

One litre of fresh Thames water was infected with 5 c.c. of a gelatine culture of virulent anthrax, the culture being one day old, and distributed into three flasks A, B, and C, in equal quantities. The flask A was aërated, as before, B stood quietly by its side: these two flasks were exposed to ordinary diffused daylight, while C stood quiet in a south window, exposed to what sunlight could fall on it in November.

At the outset, we examined the infected water, and found the proportions of organisms as follows:—Water organisms = from 370 to 450 per 1 c.c., and anthrax from 150,000 upwards per 1 c.c.

After seven days, our examination gave the following results, tabulated as averages:—

State of flask.	Number of drops in culture.	Total bacteria per 1 c.c. original.	Proportion of anthrax to other organisms.	Remarks.
Aërated 7 days....	1	4,000,000	1 : 35	The numbers were so enormous that we could make nothing of the 3-drop cultures.
Non-aërated 7 days	1	6,000,000	1 : 50	
Insolated 7 days...	1	6,000,000	?	

After taking the samples for direct culture, we placed similar samples of each flask at 60° C. for twenty-four hours, and made plates again, with the following results:—

State of water.	Number of drops used.	Number of anthrax per 1 c.c. original.	Remarks.
Aërated	1	270,000	
Non-aërated	1	153,000	
Insolated	1	117,000	
Aërated	3	140,000	
Non-aërated	3	130,000	
Insolated	3	135,000	

Here, again, we abstain from dwelling too much on the quantitative results, though, so far as they go, they suggest that aëration favours the sporification or preservation of the anthrax, while insolation—even feeble—tends to destroy the spores. But the positive qualitative result is obvious, that the anthrax if it passes over into the spore stage in these waters becomes, thereby, to a great extent removed from the direct competition with the water organisms.

Experiments on the Action of Light on Bacillus anthracis.

It is abundantly evinced by experiments that direct insolation in some way leads to the destruction of spores of *Bacillus anthracis*, and in so far the results merely confirm what had already been discovered by Downes and Blunt in 1877 and 1878.*

From the fact that an apparent retardation of the development of the colonies on plates exposed to light was observed several times under circumstances which suggested a direct inhibitory action of even ordinary day-light, the author went further into this particular question with results as startling as they are important, for if the explanation given of the phenomena observed in the following experiments turns out to be the correct one, we stand face to face with the fact that by far the most potent factor in the purification of the air and rivers of bacteria is the sun-light. The fact that direct sun-light is efficacious as a bactericide has been long suspected, but put forward very vaguely in most cases.

Starting from the observation that a test-tube, or small flask, containing a few c.c. of Thames water with many hundreds of thousands of anthrax spores in it may be entirely rid of living spores by continued exposure daily for a few days to the light of the sun, first shown for water by Straus ('Soc. de Biologie,' 1886, p. 473), and that even a few weeks of bright summer day-light—not direct insolation—reduces the number of spores capable of development on gelatine,

* See p. 237 of "First Report to the Water Research Committee of the Royal Society" ('Roy. Soc. Proc.,' vol. 51, 1892) for the literature on this subject up to 1891.

it seemed worth while to try the effect of direct insolation on plate-cultures, to see if the results could be got more quickly and definitely.*

Preliminary trials with gelatine plate-cultures at the end of the summer soon showed that precautions of several kinds were necessary. The direct exposure of an ordinary plate-culture to the full light of even a September or October sun, especially in the afternoon, usually leads at once to the running and liquefaction of the gelatine, and although the exposed plates eventually showed fewer anthrax colonies than similar plates not exposed, the matter was too complicated to give satisfactory results. Obviously one objection was that the spores might have begun to germinate, and the young colonies killed by the high temperatures.

Experiments made in October with gelatine plates wrapped in black paper, in which a figure—a square, cross, or letter—was cut, also led to results too indefinite for satisfaction, although it was clear in some cases that if the plates lay quite flat, the illuminated area was on the whole clear of colonies, while that part of the plate covered by the paper was full of colonies.

But another source of vexation arose. After the plates had been exposed to the sunlight for, say, six hours, it was necessary to put them in the incubator (20—22° C. was the temperature used) for two days or so, to develop the colonies, and in many cases it was observed that by the time the colonies were sufficiently far advanced to show up clearly, liquefaction had extended so far as to render the figure blurred and doubtful.

Stencil plates of zinc were employed with, at first, equally uncertain results. The stencil plate was fixed to the bottom of the plate culture, outside, and every other part covered with blackened paper: the plate was then placed on a level surface, the stencil-covered face upward, and exposed to the direct sunlight. As before, the gelatine softened and in many cases ran, and the results were uncertain, though not altogether discouraging.

In November it was found that more definite results could be obtained, and the problem was at last solved.

Meanwhile it had already been found possible to obtain sun prints in the following way with agar plates. Ordinary agar was heated and allowed to cool to between 50° and 60° C., and was then richly infected with anthrax spores, and made into plates as usual. Such plates were then covered with a stencil plate on the lower face—the stencil plate being therefore separated from the infected agar only by the glass of the plate—and wrapped elsewhere closely in dull

* It appears that Buchner ('Centr. f. Bakt.,' vol. 12, 1892) has already done this for typhoid, and finds the direct rays of the summer sun quite effective.

black paper, so that, on exposure to the sun, only the cut-out figure or letter allowed the solar rays to reach the agar.

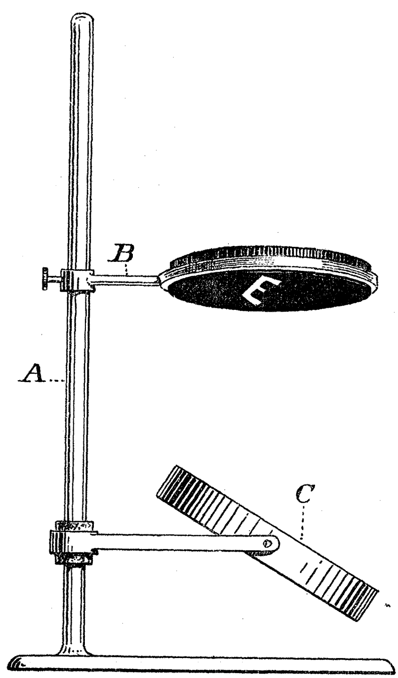
Such plates were then exposed to the direct rays of the October sun for from two to six hours; or they were placed on the ring of a retort-stand, stencil downwards, and the sun-light reflected upwards from a plane mirror below.

After the insolation, these plates were incubated for at least forty-eight hours at 20° C., and on removing the wrappers the colonies of anthrax were found densely covering all parts of the plate except the area—a letter or cross, &c.—exposed to the sun-light. There, however, the spores were killed, and the agar remained perfectly clear, showing the form of a sharp transparent letter, cross, &c., in a groundwork rendered cloudy and opaque by the innumerable colonies of anthrax.

Experiments proved that this was not due to high temperature, for a thermometer with its bulb next the insolated glass rarely rose beyond 14° to 16° C., and never beyond 18° C., and even if the thermometer did not record the temperature inside the plate, this can scarcely have been much higher.

As long as this latter point remained uncertain, however, the

FIG. 1.

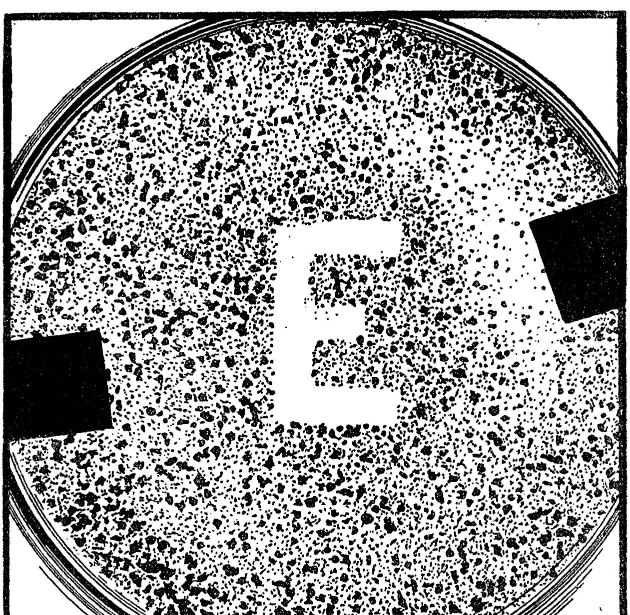


experiments could not be regarded as satisfactory; whence it was necessary to again have recourse to gelatine cultures. The gelatine employed began to run at 29° C., and in November it was found that such plates exposed outside, either to directly incident sunshine, or to directly reflected rays, showed a temperature of 12° to 13° C. at the insolated glass surface, and even five to six hours' exposure caused no running of the gelatine.

The following experiment may be selected as a type of the rest:—A (fig. 1) is the upright of an ordinary retort-stand; on the ring B rested a gelatine plate-culture of anthrax spores, covered with black paper everywhere except the cut-out letter E, seen on its lower face. C was an ordinary plane microscope-mirror, with its arm fitted to a cork on A.

The whole was placed in the middle of a field at Cooper's Hill at 9.30 A.M. on Wednesday, November 30, and exposed to the clear, but low, sunshine which prevailed that day, the mirror being so arranged (from time to time as necessary) as to reflect the light on the E the whole period, until 3.30 P.M., when the plate was removed and placed in the dark incubator at 20° C. On the following Friday —*i.e.*, after less than forty-eight hours' incubation—the letter E stood out sharp and clearly transparent from the faint grey of the rest of

FIG. 2.



the plate of gelatine. Not a trace of anthrax could be found in the clear area, even with the microscope, while the grey and almost opaque appearance of the rest of the plate was due to innumerable colonies of that organism which had developed in the interval.

It was impossible to incubate the plate longer for fear of liquefaction, whence the sceptical may reply that the anthrax exposed to light was only retarded; the experiments with agar show that such is not the case, however, and that if the insolation is complete the spores are rendered incapable of germinating at all, as proved by removing pieces of the clear agar or gelatine and attempting to make tube cultures from them: in all cases where insolation is complete they remain sterile.

The chief value of these gelatine plate exposures in November, however, is that they prove conclusively (1) that the rays of a *winter* sun are capable, even if reflected, of killing the spores, and (2) that it is really the solar rays which do this directly, and not any effect of a higher temperature, since the gelatine remains solid throughout.

Experience has shown, however, that some precautions are necessary in selecting the anthrax cultures employed for these experiments with gelatine. The light certainly retards or kills (according to its intensity or the length of exposure) virulent spores, but if one takes the spores, mixed with vegetative bacilli, direct from a thoroughly liquefied gelatine culture, or from a bouillon culture, the plates are apt to be liquefied too rapidly for the proper development of the light print, evidently because so much of the liquefying enzyme is carried in when inoculating the plates. The same danger is run when active bacilli alone are employed.

The best method of avoiding these disadvantages has been found to be the following, and it has the additional merit of enabling us to prove, beyond all doubt, that the ripe spores of *Bacillus anthracis* are really inhibited or killed by sunlight.

A few c.c. of sterile distilled water in a tube are thoroughly saturated with the anthrax spores taken from an old culture which has never been exposed to light, and the tube placed for twenty-four hours at 56° C.; this kills all immature spores, bacilli, and enzymes, and leaves us with a crop of the most resistant and fully matured virulent spores.

Experiments with such spores have been made to determine the relative power of the different rays of the spectrum to destroy the anthrax.

It is necessary to note first, however, that in experimenting with the electric light, although but few exposures have been made as yet, it is evident that its effects are feebler than those of the winter sun.

At present it has only been possible to observe that the inhibiting effects are stronger at the blue end of the spectrum than at the red,

and exposures to sunlight passing through coloured glasses confirm this result; but the observations are being continued in the hope of getting a perfectly sharp record of the effects of each set of rays.

The following series of experiments are quoted in detail, because they teach several details of importance, in addition to proving the main fact.

On December 7 three gelatine plates and five agar plates were prepared with spores from a very vigorous and virulent agar tube of anthrax. The spores, which were quite mature, were not subjected to heat, but simply shaken in sterile water to wash and separate them thoroughly.

The three gelatine plates were made at 35° C., the agar plates at 60° C., neither of which temperatures could injure the ripe spores.

The three gelatine plates were labelled *p* 1, *p* 2, and *p* 3, and the agar plates *p* 4 to *p* 8 in order.

Immediately after making the plates, all were exposed to the December sun, except plates *p* 4, *p* 5, and *p* 6, and this was done as follows:—In each case the plate had a stencil plate with a cut-out letter on its lower face, and arranged as described above (p. 304).

p 1, a gelatine plate with a *large* letter M, was exposed, face down, to the light reflected from a mirror (see fig. 1) for three hours on December 7, and for four hours on December 8, the interval being passed in a cold room (*t* about 8—9° C.), and then incubated at 20° in the dark.

p 8 was treated in exactly the same manner. But this was an agar plate with a *large* W.

p 2, a gelatine plate with a *large* H, was exposed and treated in the same way, except that no mirror was used, the latter being upwards towards the sun.

p 3, a gelatine plate with a *large* B, was similarly exposed, face up, but a plane mirror arranged to reflect light down upon it.

p 7, an agar plate with a *large* E, was treated exactly as the last.

There now remain the three agar plates, *p* 4, *p* 5, and *p* 6, to account for.

p 4 was placed forthwith in the dark incubator at 20° C.

p 5 and *p* 6 were kept for eighteen hours in a drawer, the average temperature of which is almost 16° C., and were not exposed till next day (December 8), when they lay for five hours, face upwards, and with a mirror above them. *p* 5 had a *small* E, and *p* 6 a broad but small I, to let the light in.

After exposure, these also were put in the same incubator with the others.

Nothing was visible to the unaided eye on these plates (except *p* 4) until the 11th instant, though the microscope showed that germination was proceeding on the 10th. The plate *p* 4, however, had a

distinct veil of colonies all over it on the 9th, and this had developed to a dense typical growth by the 11th.

On December 11, at 10 A.M., the state of affairs, as regards the exposed plates, was as follows:—

p 5 and *p* 6 showed each a sharp transparent letter—E and I respectively—of clear agar in a dull grey matrix of strong anthra2 colonies, which covered all the unexposed parts of the plate.

p 1, *p* 2, and *p* 3 showed in each case a perfectly clear central patch, about $1\frac{1}{2}$ inches diameter, with anthrax colonies in the gelatine around. These anthrax colonies were the *larger and more vigorous the more distant they were from the clear centre.* In other words, the anthrax spores had begun to germinate, and the colonies were growing more vigorously, in centripetal order.

On *p* 7 and *p* 8 germination was beginning, but the colonies were as yet too young to enable one to judge of the results.

The first point of interest is to account for the pronounced results in the plates *p* 5 and *p* 6, and the want of sharp outlines in *p* 1, *p* 2, and *p* 3, and the explanation seems to be that, owing to the plates 5 and 6 having laid over night at 16° C., the spores began slowly to germinate out, *and were consequently in their most tender condition* when exposed to the sunlight next day.

The peculiar centripetal order of development of the colonies on plates *p* 1, *p* 2, and *p* 3 gave rise to the following attempt at explanation. After observing that the clear space in the middle was not due to the centre of the plate being raised, and the infected gelatine having run down to the periphery—a possible event with some batches of Petrie's dishes—it was surmised that the *large* letters employed might give the clue.

This was found to be the case. The solar rays on entering the plate were largely reflected from the glass lid of the plates, and so produced feebler insolation effects on parts of the plate around the letter: these effects were naturally feebler and feebler towards the margin, and so the inhibitory action became less pronounced at distances further and further removed from the centre. Those spores, therefore, which were nearest the periphery germinated out first, and those nearer the centre were retarded more and more in proportion to their proximity to the insolated letter.

That this is the correct interpretation of the facts follows clearly from the further behaviour of the above plates.

At 10 P.M. on the 11th—*i.e.*, twelve hours after the morning examination—the plates *p* 1, *p* 2, and *p* 3 exhibited their respective letters M, H, and B quite clearly, in the grey matrix of anthrax which had rapidly developed in the interval, and excepting a sligh8 want of sharpness in the H of *p* 2, the results could hardly have been more satisfactory.

In *p* 7 and *p* 8 the *very faint* outlines of the letters were also showing.

On the 12th, at 8.30 A.M., the gelatine plates had begun to run, and although the M of *p* 1 was still intact, and very well marked, *p* 2 had liquefied completely, so that the H was a clear patch with blurred outlines in the centre. *p* 3 still showed the outlines of the B, but it was impossible to keep it longer.

The main point was definitely established, however, and the treatment of the plates proves conclusively that the spores are not killed by high or low temperatures, *but by the direct solar rays.*

These experiments are being continued in order to answer some other questions in this connexion.

The gelatine and agar after such exposures as have been described are still capable of supporting a growth of *B. anthracis* if fresh spores are sown on them, whence the effects described are not merely due to the sub-strata being spoilt as food material.

That the action of the light is *direct* on the spore, and not due to any reaction from the medium, I have recently shown by the following new method:—

A thin layer of *dried spores only*, spread on glass without food materials, shows the letter as in the experiments on pp. 305—306, *if a slab of solidified agar is placed on the film of spores after exposure*, and the whole incubated; whereas the reciprocal treatment—where the *agar alone* is exposed, and then laid on a *film of spores*—yields negative results, the spores germinate equally well all over.*

Conclusions to Part II.

The following conclusions are to be drawn from the results of the experiments recorded in this Part II of the Report:—

1. Thames water, like all open waters, contains a variable number of bacteria at all times (pp. 244—256).

2. The actual numbers of these bacteria are not great, but comparisons show that there are more in the Thames in December than in March, and fewer still in June (pp. 246, 252, 254).

3. There are no reasons for supposing any of these water bacteria to be pathogenic, and some of them have been recognised as known saprophytes (pp. 247, 280, 285—290).

4. In agreement with the universal experience of those observers

* This proof that the action of the light is *direct* on the spores is opposed to Roux's conclusions ('Ann. Inst. Pasteur,' 1887, pp. 445—452), and in support of those of Arloing ('Compt. Rend.,' vol. 104, 1887), and of Janowski ('Centralbl. für Bakteriol.,' 1890, Nos. 6—8). These authors worked with less perfect methods, however, and Janowski's results concern typhoid only. I have given more extensive results in the paper read to the Royal Society on February 16, 1893.

who have attended to the question, we find these water bacteria to multiply with astounding rapidity if the water is allowed to stand for a few days; the maximum numbers are reached in from one to four days as a rule, and the curve itself is exceedingly steep and sharp (pp. 249—256).

5. Our observations go to show that the rapidity of increase and the maximum numbers reached depend on various factors—temperature, oxygen supply, the amount and quality of the food materials in the water, and the nature and numbers of the bacteria concerned (pp. 254, 291, 295, 299—302).

6. We have experimented with the waters, chiefly of the Thames, in three conditions, viz.:—(1) fresh from the river and not subjected to any treatment; (2) deprived of all of the above bacteria by filtration through porous porcelain; and (3) sterilised by heat. (4) Experiments have also been made with distilled water. We regard the four conditions of water referred to as essentially different one from another (pp. 259—263, 266—273, 275—278).

7. We have employed such waters to test the power of resistance of *Bacillus anthracis* (anthrax) both in the form of spores and of vegetative bacilli, and also in the asporogenous state (pp. 256—285).

8. We have, moreover, employed the anthrax in a virulent and in a weakened condition (pp. 266—273).

9. And we have experimented under various conditions as regards (1) time, (2) temperature, (3) light, (4) the presence of other organisms, with the following results briefly summarised:—

10. Neither as spores nor as bacilli—weak or strong—is anthrax killed forthwith in any of the waters under any of the conditions tested; but the spores are immensely more resistant than the bacilli (pp. 259—285).

11. In the dark, and at moderate temperatures, the spores of anthrax retain their powers of germination and infection for many months—we have proved up to eight months—in any of the waters referred to (pp. 278—283).

12. In direct sunlight, however, the spores in the waters undergo rapid destruction, depending on the intensity of the insolation and the time of exposure (pp. 279, 283, 303).

13. That this destruction is directly due to the light-rays, especially at the blue end of the spectrum, and not to a rise of temperature fatal to the spores, is definitely proved by the experiments with gelatine and agar plates. These experiments also demonstrate conclusively that the bactericidal action is really *direct*, and not due indirectly to the action of the solar rays on the medium (p. 310).

14. The value of the experiments on the bactericidal action of direct sunlight on the spores of *B. anthracis* is the more important when it is reflected that the most decisive results have been obtained

by exposure to the rays of a *winter sun* (November and December), at temperatures so low that no question of heat can come into consideration; moreover, the experiments prove that the bacteria spores are really killed, and not merely retarded (p. 307).

15. That we have here an essential part of the explanation of numerous phenomena cannot be doubted, *e.g.*, the purity of shallow running waters, and the steady diminution of bacteria from our rivers, lakes, &c.; while, conversely, the suspension of solid particles, rendering water turbid, may obviously react on their bacterial life by intercepting the sun's rays.

16. These experiments also suggest how necessary it is to conduct all cultures of such bacteria in the dark; and bring vividly before us the importance of direct sunlight in our streets and dwellings, &c., and in numerous circumstances of life.

17. In no case have we succeeded in showing that *Bacillus anthracis* multiplies to any considerable extent in the form of vegetative bacilli in the above waters, unless appreciable quantities of organic food materials are added and the temperature is raised to above 12° C. So far as we can decide, the bacilli either die off in the course of the first day or two, or, if the conditions are favourable, they form spores (pp. 272—273). I regard this question as to the power of the bacilli to multiply or form spores in the water as the most important from a hygienic point of view; unfortunately it is also by far the most difficult one to answer.

18. Aëration and consequent disturbance of the water containing anthrax does not destroy nor appreciably affect the latter (pp. 299—302). Nor does the presence of ordinary green Algæ in the standing water seem to affect it, or, at any rate, not more than can be explained by the diffused light necessary for the Algæ (pp. 278—283).

19. Experiments prove that anthrax by no means succumbs easily, if at all, in the struggle for existence with *Bacillus fluorescens liquefaciens*, one of the commonest of the Thames bacteria, and remarkable for its aërobism and liquefying powers (pp. 290—298).

20. Whether the result will be the same with other bacteria selected for antagonism remains to be shown; several other Thames bacteria have been isolated, and are being studied in detail to the end that their effects may be tested.

Part III.

Joint Conclusions arrived at by both Authors.

After carefully comparing the results, we beg to submit to the Committee the following conclusions at which we have arrived on the subject of inquiry:—

1. The waters both of the Thames and of Loch Katrine normally contain a number of different forms of micro-organisms, some of which have been isolated and described (pp. 178—180, 186—191, 244—246, 285).

2. These bacteria are, as far as our comparisons have been pursued, more numerous in the water of the Thames than in that of Loch Katrine, and the numbers in the Thames water at least have been shown by us to be subject to well-marked seasonable variations, being usually much greater in winter than in summer (pp. 178—180, 246). This relationship is probably due to the Thames water in dry weather being to a large extent derived from springs, whilst after rain, especially in winter, it receives considerable accessions of surface water rich in bacterial life and the organic materials which promote the growth and multiplication of micro-organisms (pp. 178—180, 225).

3. Hitherto no pathogenic bacteria have been found in the Thames water, either by other observers or ourselves (p. 258).

4. In agreement with the universal experience of all observers who have given attention to the subject, we have found that the water bacteria, both of the Thames and of Loch Katrine, multiply with astounding rapidity when these waters are allowed to stand for a few days, a maximum being rapidly reached, which is followed by a corresponding, although less precipitate, decline (pp. 190, 191, 226, 249, 254.)

5. An adequate explanation of this remarkable multiplication has not yet been given, and is the more difficult to find, inasmuch as it has been shown that the same phenomenon occurs in the case of waters, like those of deep wells, which are almost wholly destitute of organic matter. Again, although oxygenation and a high temperature undoubtedly accelerate this multiplication, it even takes place to a surprising extent at the low temperature of a refrigerator.

6. We have experimented with the waters both of the Thames and Loch Katrine in the three following conditions :—(1) in their natural state as derived from the river and loch (p. 246); (2) sterilised, or

deprived of all their bacteria, by filtration through porous porcelain; and (3) sterilised by heat, 100° C.; (4) experiments have also been made with distilled water. We regard these several conditions of the waters referred to as essentially different one from another (pp. 184, 185, 213, 214, 228).

7. Into such waters we have introduced the *Bacillus anthracis* (anthrax) in the form of (*a*) vegetative bacilli (pp. 256—278); (*b*) spores (p. 278); (*c*) in the "asporogenous" variety (pp. 274—278); contrasting also the effect of using large and small quantities of this micro-organism, and of employing it in a virulent and an attenuated or weakened condition respectively (pp. 184, 213, 228).

8. The principal factors to which we have devoted attention in these experiments have been (1) the temperature at which the infected waters were maintained; (2) whether they were exposed to light or preserved in darkness; (3) the presence or absence of other organisms besides anthrax in the waters (pp. 184, 214, 228; all experiments were conducted in the dark, excepting when otherwise stated, 40, 45, 54, 57, 59, 67, 69, 71; 92, 119, 126—134).

9. We will in the first instance call attention to the results which we have obtained in our experiments with these spores.

We found that the behaviour of the spores was very different, according as they were introduced into the unsterilised or sterilised waters respectively (pp. 181—243).

10. In the sterilised waters their behaviour was practically uniform, irrespectively of whether Thames or Loch Katrine water was employed, irrespectively of whether the water was sterilised by filtration through porcelain or by steam, and also irrespectively of whether the waters were preserved at a summer temperature of 18—20° C., or in the refrigerator at 4—9° C. In all cases the spores retained both their vitality and their virulence for many months. After this prolonged residence in these sterile waters, they were recognisable by cultivation in either the same or in only slightly diminished numbers from those in which they were originally introduced into these waters. These infected sterile waters, after standing for upwards of seven months, were also invariably fatal to the animals into which they were inoculated (pp. 200, 219, 232, 234, 238).

11. The same results with these infected sterile waters were obtained irrespectively of whether they were preserved in absolute darkness or freely exposed to diffused daylight. Direct sunshine, on the other hand, was rapidly fatal to the anthrax spores in these waters within 84 hours. In the waters so insolated anthrax could not be detected by cultivation, and animals inoculated with these waters remained alive. But in order to make absolutely certain that the anthrax spores were quite extinct in these insolated waters, we incubated them with some sterile broth, so that if only a single spore had

remained in the water it would have multiplied abundantly; but the waters, even after this treatment, proved innocuous to animals (pp. 209—212).

12. The striking results obtained by direct insolation at low temperatures in the open air in winter bring vividly before us the extreme importance of this bactericidal action of direct sunlight, for they show conclusively that the action is direct, and not due to any rise of temperature from the heat rays (pp. 303—310). Other facts and their consequences are given in the conclusions to Part II.

13. We found that when the spores were introduced even in very large numbers into unsterilised waters in their natural condition, they were often no longer recognisable by the ordinary cultivation methods after the lapse of a few days, and it was only by resorting to special methods of detection that the anthrax spores could be discovered. By employing these special methods, however, we have conclusively shown that the number of anthrax spores undergoes a continuous decline in such unsterilised waters, and thus presents a marked contrast to the persistence of the numbers in the sterile waters referred to above. Notwithstanding this decline in the number of anthrax spores, their presence could still be demonstrated many months after their introduction into the Thames water, and this infected water still retained its power of killing animals after upwards of seven months (pp. 278—283), either by direct inoculation (when large numbers of anthrax spores had been originally introduced) or after preliminary incubation with sterile broth (which had to be resorted to when only a small number of anthrax spores was originally introduced, pp. 192—200, 214—219, 229—231, 236—238).

In the case of the Thames water we found but little difference in the result when the waters were kept at winter and summer temperatures respectively, but in the case of the Loch Katrine water a marked difference was exhibited in this respect, for at the summer temperature (18—20° C.) the anthrax spores underwent such rapid degeneration that after three months they were no longer recognisable by cultivation. Moreover, the water kept at the summer temperature proved in every case to be no longer fatal to animals when inoculated directly, and out of two such specimens of water which had been specially incubated with broth for the purpose of revivifying any lurking anthrax spores that might remain, only one became virulent, showing that in the other at least complete extinction of the anthrax spores had taken place. It is suggested that this comparatively rapid destruction of the anthrax spores in unsterilised Loch Katrine water at 18—20° C. is due to the elaboration of bactericidal products by the water bacteria, and not to the character of the moorland water itself, for in the sterile Loch Katrine water the destruction of anthrax spores at this temperature did not take place (pp. 228—239).

14. The results obtained with the anthrax spores in the unsterilised waters were not influenced by whether these waters were preserved in darkness or exposed to diffused daylight. Exposed to direct sunshine, however, the anthrax spores were rapidly destroyed, not *more* rapidly, however, than in the sterilised waters under the same exposure (pp. 204—213).

15. In experiments made in order to test the nature of the conflict between anthrax and particular forms of water bacteria, the *Bacillus fluorescens liquefaciens* (Flügge) was employed in pure cultivation along with anthrax in approximately equal proportions. The results, however, show that this saprophyte, at any rate, has not the power of rapidly destroying the anthrax spores ; indeed, there was no evidence that either it, or its products, act prejudicially on anthrax spores at all (pp. 290—298).

16. In connexion with the antagonistic interests of the anthrax on the one hand and the several kinds of water organisms on the other, it is worthy of note that in one experiment in which anthrax spores were introduced into unsterilised Thames water exposed freely to daylight, and in which in addition to water bacteria there was also present a quantity of small algæ, the anthrax spores survived the conflict with these competing forms for upwards of seven months, although enormously reduced in numbers and much impaired in virulence (pp. 278, 283).

17. To summarise our results with anthrax spores in one sentence, we may state generally that there is one natural agency at least which is capable of destroying them in surface waters to which they may have gained access, viz., the action of direct sunshine on the organism. Whether the activity of water bacteria may be added as a second bactericidal agent is not definitely determined, but, in any case, of these two influences the sunshine is by far the more rapid and the more potent, though its sphere may be much more restricted.

18. *Behaviour of Anthrax Bacilli.*—As regards the behaviour of anthrax bacilli free from spores, it should be pointed out that we have only experimented with such spore-free bacilli obtained from artificial cultures, and not with those derived directly from the organs of an animal dead of anthrax. We have in many cases found that the bacilli obtained from artificial cultures behave in essentially the same way when introduced into water as do the spores under the same circumstances, and apparently for the reason that the bacilli introduced rapidly produce spores in the water, and the subsequent phenomena thus become identical with those which we have already discussed above (pp. 260, 272, 278).

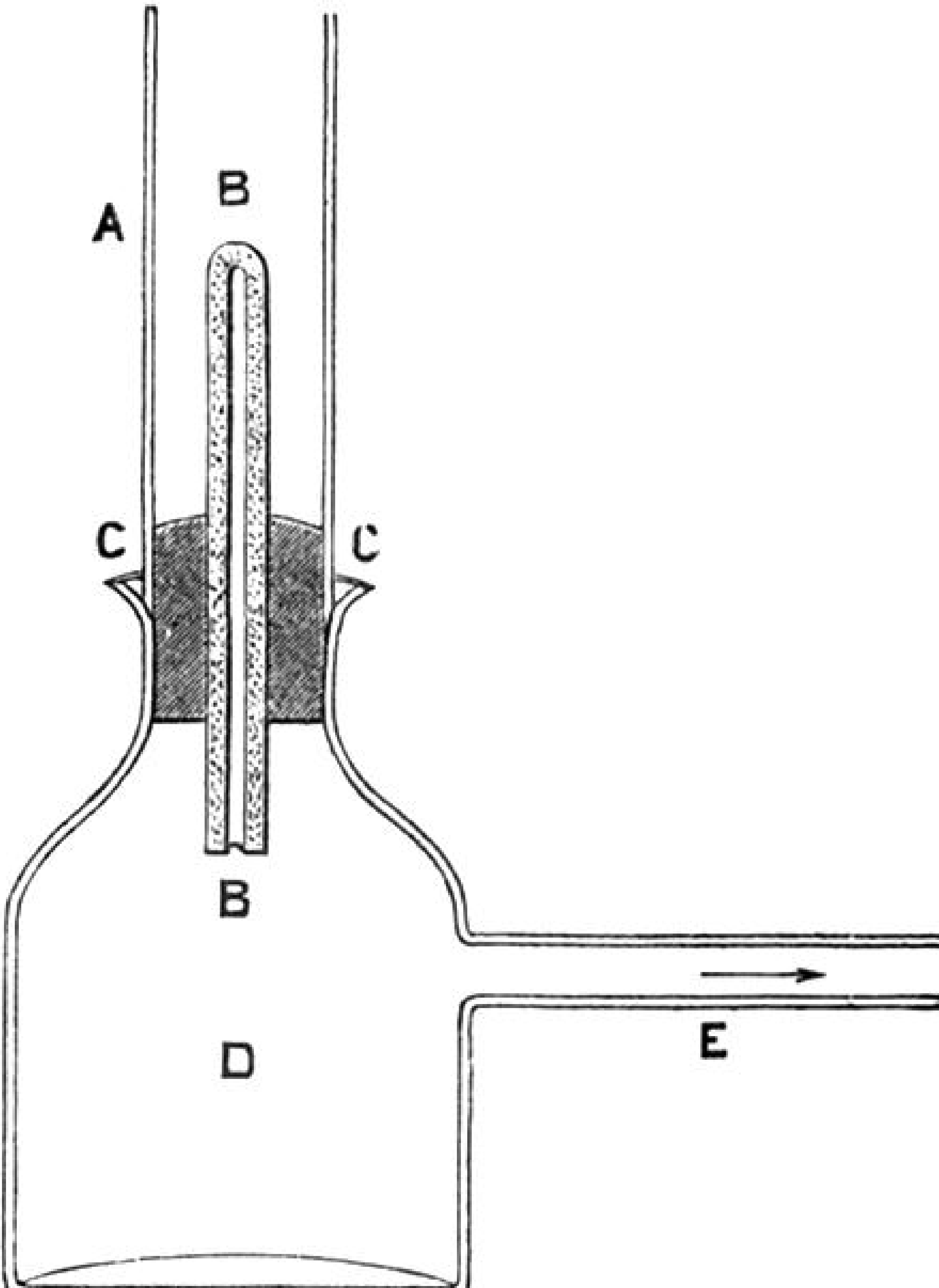
19. Some of the evidence points to the possibility of the multiplication of the bacilli in waters containing more than the usual amount

of organic materials; but in no case does it support the view that *Bacillus anthracis* can live and multiply like a water bacterium in ordinary waters (p. 273).

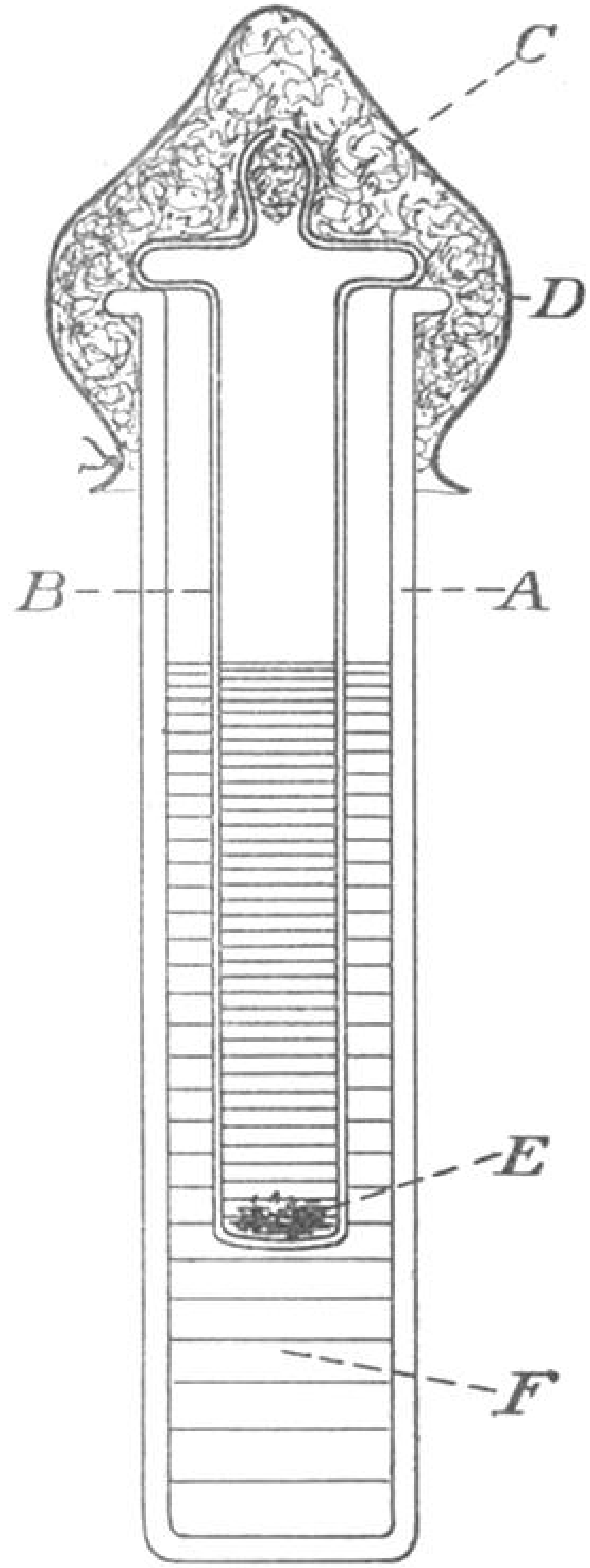
20. As regards the behaviour of that variety of anthrax bacillus which is known as "*asporogène*," and which is incapable of forming spores under any known circumstances whatever, our experiments are not yet sufficiently advanced to warrant any conclusions being drawn from them at present (pp. 274—278). The great manipulative difficulties of experiments with spore-free bacilli have already been pointed out, and no one has, so far as we know, as yet, overcome them.

In conclusion, we would point out that the chief hygienic interest of our investigation is centred in the behaviour of the anthrax spores, which, as we have already pointed out, may be regarded as representative of the extreme limit of endurance possessed by pathogenic bacteria; on the other hand, the most important question to be examined was whether the bacilli of anthrax can grow and multiply or form spores in such waters, and our results point to this being possible only under special conditions. We trust, therefore, that the information which we have collected, both from our own experiments and from the published results of other observers, concerning the behaviour of these hardy anthrax spores, may serve as a basis for practically assessing the higher limit of possible vitality which may be exhibited by pathogenic micro-organisms gaining access to potable water.

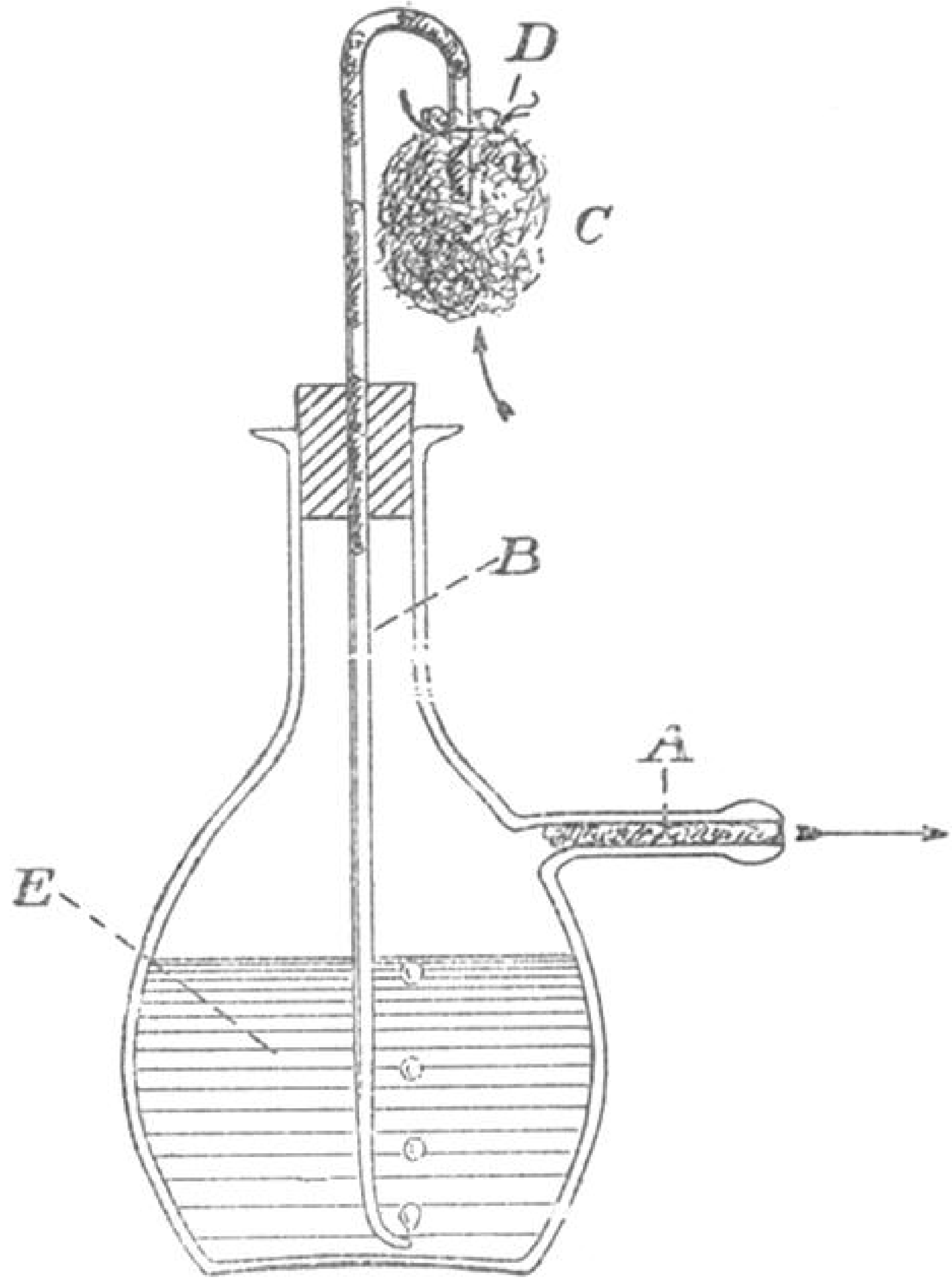
FIG. 1.



Porcelain Filter.



A, glass cylinder; *B*, Chamberland filter; *C*, sterilised cotton wool; *D*, sterilised paper cap tied over the cotton wool; *E*, *Bacillus fluorescens liquefaciens*; *F*, *Bacillus anthracis*.



A, lateral tubulure plugged with cotton wool and attached to pump; *B*, curved tube plugged at intervals with cotton wool to filter air which passes into liquid below; *C*, large wad of cotton wool, tied on to air tube at *D*; *E*, water containing bacteria to be experimented with.

FIG. 1.

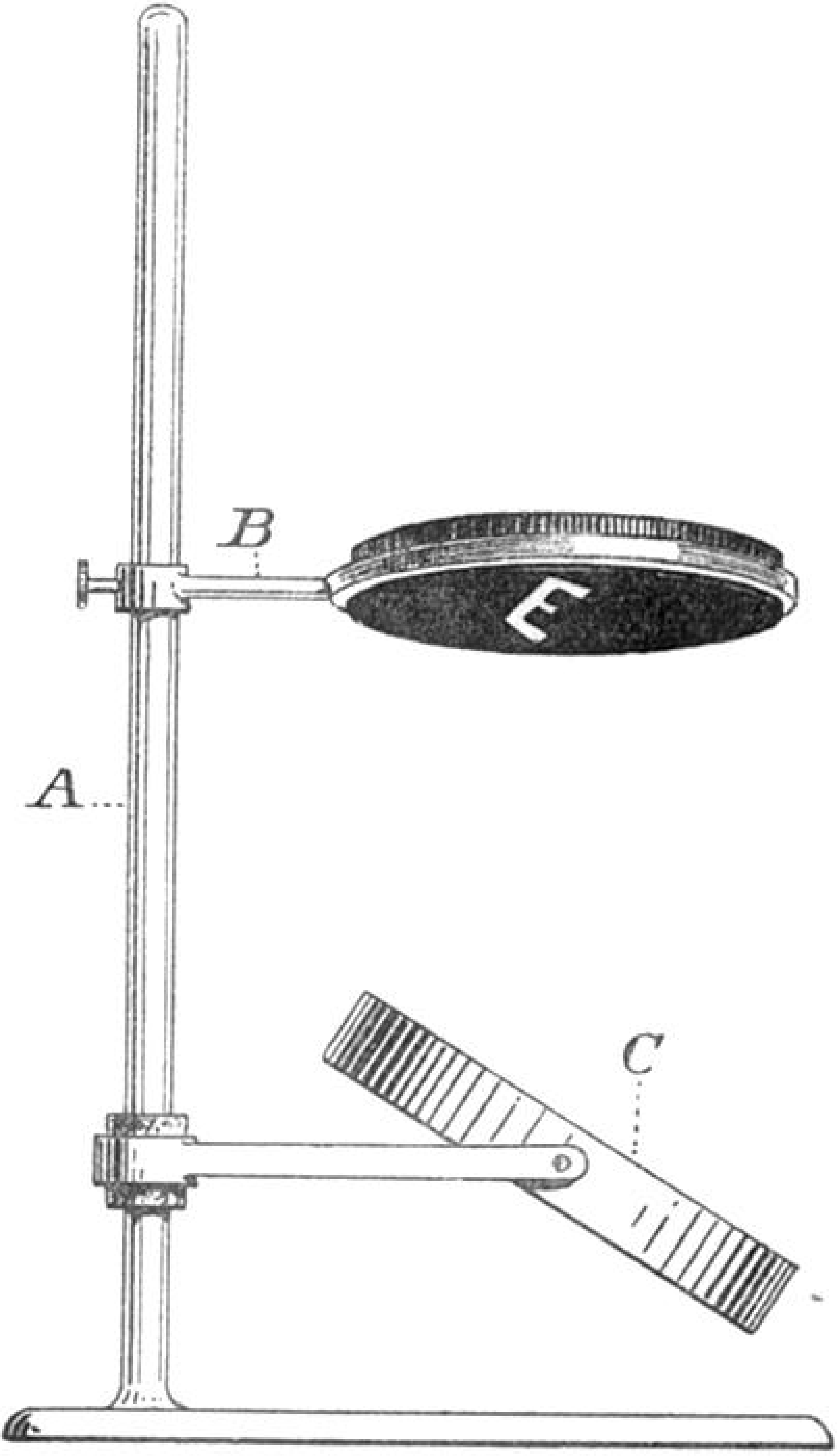


FIG. 2.